Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Annual Report 2022

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NICO 2022 by the numbers

10
Research Groups
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BRIEF HISTORICAL NOTES

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

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

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

On January 28 and 29, 2016 a Panel of external reviewers (P. Alves, Lisbona, M. Bentivoglio, Verona, M. Celio, Fribourg) visited NICO. After evaluating activities and researcher of NICO during the first five years, they issued a report, which is available on the NICO website. On January 13 and 14, 2020, the new international Advisory Scientific Committee made a second onsite visit: their report maybe found at the link https://www.nico.ottolenghi.unito.it/eng/Institute/Scientific-report .

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.

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

THE COLLABORATIVE VISION AT NICO

Since its foundation in 2010, 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 centre for Neuroscience (called Neuroscience Institute of Turin – NIT), which gathers most researchers active in the field in Turin (even outside the University). NICO researchers are part of doctorate schools (Neuroscience, Bioengineering and Medical-Surgical Sciences, Molecular Biotechnology, Complex Systems for Bioquantitative Medicine and Veterinary Medicine) of the University of Turin and of the National doctorate in Sustainable Development and Climate change) of the IUSS (Pavia), and hosts 24 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. In particular, NICO researchers are directly involved in the organization of the master degree in Biotechnology for Neuroscience (of prof. Di Cunto and Boido are respectively president and vice president).

Currently, NICO laboratories host 50 students who are developing their Bachelor/Master thesis projects and stage.

NICO collaborates with several other research centres of the University of Turin, such as the Molecular Biotechnology centre, the IRCCS Candiolo and the Brain Imaging Centre.

NICO members belong to the Departments of Neuroscience, Clinical and Biological Sciences, Veterinary Sciences and Systems Biology. NICO members belonging to the Departments of Neuroscience and Veterinary Sciences of UNITO have participated in the projects, which were awarded by the MIUR Departments of Excellence 2017-2022. The Departments of Neuroscience and Clinical and Biological Sciences were awarded departments of excellence at the end of the 2022 for the 2023-2027 period. 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, the microscopy facilities at the NICO are part of the Open Access lab program of the University of Turin, and recently European project of the University of Turin RE-UNITA (workpackage 4). Within this frame, in 2020 the microscopy facilities at NICO were reorganised to create the Platform for Imaging Cavalieri Ottolenghi, PICO, https://www.nico.ottolenghi.unito.it/eng/PICO-).

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. In addition, 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 Neuroendocrinology 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, 2019 and (virtual) 2021 editions were organized with the administrative help of the Ottolenghi Foundation.
In 2022, Prof. Boido and Dr. Stanga organised an international workshop on motoneuron diseases which was attended by 115 people.
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. From January 2022 the NICO scientific director, A. Vercelli is acting as president of the Italian Society for Neuroscience. The National meeting of the Society in 2023 (750 attendees foreseen) will be held in Torino and Prof. Di Cunto serves as president of the local organizing committee.
The Clinical Neurobiology group organises local and national meetings on multiple sclerosis at the San Luigi Hospital.
NICO has been credited by MUR (Ministry of University and Research) in the list of the Italian Private Research Institutes for the first time in 2015 and thereafter (last time on December 2021).
Following the suggestions of the international Advisory committee to improve the international interactions of the NICO, from January 2022 NICO has hired a consultant for internationalization, Dr. Mariasilvia Ciola. In 2022 this lead to informal contacts with other institutes such as the Cajal Institute of Madrid, the Department of Neuroscience of the University of Rio de Janeiro and with Israeli institutions. For the following year (2023) it has been programmed a visit to several Universities in Japan (University of Tokyo, University of Osaka and Riken) and to Brazil (San Paolo and Rio de Janeiro) to explore the possibilities of joint projects and collaborations.
Dr. Ciola also proposed and organised the NICO participation at AIRI, the Italian Association of Industrial Research, which is the network of the main enterprises committed in R&D (e. g. pharma, bio-medical, high-tech). Moreover, in June 2022 she was instrumental to the organisation of a webinar sponsored by the Italian Embassy in Tokyo, gathering NICO's researchers and two eminent neuroscientists from the Riken Institute and the University of Kyoto. This webinar laid the foundations for future collaborations with these two prestigious institutions. During the two last months of the year, Dr. Ciola collaborated in several fund raising activities regarding the Conference of the Italian Society of Neuroscience.
Researchers at NICO participate to the PNRR projects in the frame of the European "Recovery Europe" D34H (digital and biological twin of the patient).

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 behavioural 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.

NICO AS A GREEN LAB

NICO and its researchers are strongly committed in the actions related to the European Green Deal.
First, its researchers are performing several projects related to the effects of pollutants on the nervous system and, on the reverse, on the beneficial effects of the green environment on brain health. Second, they participate to the new national doctorate school. Third, they are involved in the dissemination on these knowledges (see the organisation of the show "The mountain touch" in collaboration with the Museum of Mountains in Torino and the Italian National Institute of Health). Fourth, together the Council of Administration, the director is preparing a plan of reduction of energy usage. Finally, a

committee has been nominated to suggest policies and activities to change the attitude of the people and participate to cleaning of the environment.

NICO AS PART OF THE ALBA NETWORK

The Institute has signed the ALBA declaration on equity and inclusion in science. From the website of the ALBA network: "Members of underrepresented groups face persistent barriers to equitable representation in science, technology, engineering and mathematics (STEM), particularly at advanced stages. Although the historical basis for and manifestations of underrepresentation vary by group, discipline, and region, there are striking commonalities in the result – an apparent 'leaky pipeline' that drains the talent pool. The cost of this loss of talent is high – for individuals, for research, and for society as a whole. ALBA is a network of brain scientists committed to fostering fair & diverse scientific communities. We have drafted this document as a resource for concrete, positive, evidence-based actions that individuals and organizations at any level can take to promote equity & inclusivity. We focus specifically on two contributing factors to perpetual underrepresentation in STEM: implicit bias & workplace culture. We believe that adopting the actions below will benefit all members of the research community and the scientific enterprise itself."

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 up to June 2024). 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 **ten groups**:
Adult Neurogenesis (PIs Luca Bonfanti and Paolo Peretto)
Ageing and Alzheimer's disease (PI Elena Tamagno)
Brain Development and Disease (PI Alessandro Vercelli)
Clinical Neurobiology (ff the Director)
Embryonic Neurogenesis (PI Ferdinando Di Cunto).
Nerve Regeneration (PI Stefania Raimondo – formerly S. Geuna)
Neuroendocrinology (ff PI Stefano Gotti)
Neurophysiology of Neurodegenerative Diseases (PI Filippo Tempia)
Neuropsychopharmacology (PI Carola Eva)
Physiopathology of Stem Cells (PI Annalisa Buffo)

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, and a consultant for internationalization, Dr. Mariasilvia Ciola.

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

- **University staff**: 5 full professors, 13 associate professors, 8 university research assistants, 2 technicians, 21 post-docs/bursaries and 24 doctoral students;
- **Hospital staff**: 1 manager biologist, 2 specialists in Clinical Biochemistry, 3 post-doc fellows, 1 laboratory technician.

About 50 graduating students of Biology, Biotechnology, Medicine and Psychology perform experiments for their thesis at the NICO.

Labs and Equipment

Molecular and cellular neurobiology, Neuroanatomy

The laboratory is equipped with several excellent quality research light microscopes, in particular, two confocal microscopes (Leica SP5 and Nikon) and a Nikon ViCo system, an Axioscan Zeiss slide scanner and an UntraMicroscope LaVision/Miltenyi Biotec. 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 housing and breeding, spaces dedicated to behavioural tests and, finally, rooms equipped for surgery on rodents. The laboratory for behavioural tests is equipped with mazes and infrared cameras for the behavioural analysis of locomotor activity, anxiety, depression and memory. There is also a computerized video analysis (Ethovision XT video track system) to analyse scanned images of behavioural tests. Finally, dedicated spaces, equipped for P2 procedures are available to use viruses of the corresponding biosafety level and to inject them in animals.

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 devoted to human pluripotent cell derived 2D and 3D models has recently been implemented.
In addition, NICO provides expertise and services related to molecular biology techniques, such as the preparation and analysis of proteins, DNA, RNA and microRNA. The instrumentation of molecular biology platform includes a semi-automatic system for the purification of nucleic acids, three machines for Real-Time PCR, an electroporator for bacteria or ES, as well as many other instruments as a standard laboratory for extraction and analysis of DNA, RNA and proteins.

Electrophysiology
The laboratory of neurophysiology provides tools for the preparation of micro-sections of nervous tissue that can be maintained in vitro for several hours. There are two experimental stations for patch clamp recordings of membrane potential or ionic current of single neurons in sections. These positions

are furnished with complete tools for the electrical stimulation of the axons and for application of pharmacological substances. They can also make extracellular recordings to study synaptic plasticity.

Clinical Neurobiology Laboratory (CNL)

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

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

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

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

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

Common services

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

Updates in 2022

New equipment

In 2019/2021 the Neuroscience Institute Cavalieri Ottolenghi, which participated with its members of the Department of Neuroscience Rita Levi-Montalcini to the successful Department of Excellence project, could significantly implement its instrumentation.

In 2022, we implemented the surgery and dissection rooms, by acquiring a new gaseous anaesthesia system, a new digital stereotaxic apparatus and a stereomicroscope for dissection.

Moreover, the Incucyte system (equipped by Cell by Cell, Neurotrack, spheroid and ATP metabolism modules) is now available in the cell culture facility, to perform morphological and physiologically relevant analyses (including cell health and proliferation, cell function, cell movement and morphology, and assays for complex 3D models such as organoids).

Finally, an electrophysiology setup has been built to allow in vivo recordings on both anesthetized and awake mice. The system allows to collect electrical activity from single neurons by performing in vivo single-cell patch clamp recordings, as well as to characterize network population activity collecting the local-field potential and multi-unit signals. The system is compatible with several commercial amplifiers, allowing for further implementation in the future. Currently the setup is used to record the electrical responses to auditory and olfactory stimulation on awake head-restrained mice. To this aim, an olfactometer and a sound playback system have been integrated into the system in order to precisely deliver and simultaneously record neuronal responses to odors and sounds.

In 2022 the Department of Neuroscience, following a call for grants for instruments by UNITO, applied with a project to by a 3D EM microscope to be located at NICO. The application was successful, and the procedures for the University tender was assigned to Zeiss company, for the GEMINI 600 microscope. The microscope is expected to be delivered in summer 2023. Meanwhile, the Department of Neuroscience was awarded again the project of excellence, for the period 2023-27, and this will allow further improvements in the instrumentation in the next years.

Personnel
New personnel were recruited by the University Departments collaborating at NICO: he number of Associated Professors and Research assistants has increased.
Dr. Capobianco, serving as PI of the group of Clinical Neurobiology, moved to the S. Croce hospital in Cuneo; he is still collaborating with the group. The scientific director has taken the formal direction of the group.
The contract with Charles River has just been renewed by the University of Torino for the animal house. One of the two technicians of NICO working in the facility was allocated to other duties as lab technician.

Obituary
In July, our community was deeply struck by the loss of Prof. Giancarlo Panzica. Giancarlo, a retired professor of UNITO and a former director of the Department of Neuroscience, worked for NICO and with his collaborators until the very last days of life. Even though he could not come at the NICO personally, he continued to meet people online and wrote several papers. While we mourn his death, we will remember him in future official events as an eminent scientist, a mentor and an inspiring colleague.

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, within the frame of the PNRR project D34H.

Some considerations regarding research funding
Members of the NICO have raised in the year 2022 around 1 million € in grants for UNITO. Moreover Alessandro Vercelli is local coordinator for the PNRR project D34H, for which UNITO is receiving 4.3 M€. The members of the Department of Neuroscience who work at NICO actively participate in the project of Excellence of MUR who was awarded at the end of 2022 and will be effective in 2023-2027 for an overall grant of around 7 M€, in the field of Basic Neuroscience: the project was written by A. Vercelli.
The actual agreement between UNITO and FCO foresees a contribution from UNITO to FCO of the 50% of the running costs of NICO from UNITO. Members of UNITO working at NICO apply for the governmental funding though UNITO (which is relevant to the ranking of UNITO in Italy, and of the Departments with members affiliated to NICO among the other Departments.
In addition, when possible, members of NICO apply directly to agencies though FCO administration. This led to a certain amount of grants directly administered by FCO: the relative amount of grants was very low in 2019 (33.000 €), and is increased significantly in the following years (160.000 € in 2022) notwithstanding the reduction of activity of the Clinical group due to the retirement of the PI. The agreement with the San Luigi hospital foresees a yearly contribution of 25.000 € to FCO. We foresee further increases due to the involvement of NICO in PNRR grants as a private entity.

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.

Dissemination activities in 2022
- Unistem Day (11 March);
- Video and live streaming dedicated to research on ageing at the NICO organized by Giovedì Scienza (Torino) (10 March);
- Olympic Games of Neuroscience (March 19);
- Brain Awareness Week (March 14-20)
- Science Weeks (March to May);
- Two "Open Days at NICO" (April-September)
- Researcher's Night UNIGHT (September 30)
- Science Festival of Genoa (October 22)

NICO is engaged in scientific **activities dedicated to high school students** - Olympic Games of Neuroscience and Unistem Day, national and international – as well as to general public (Researchers' Night 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 establishing 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 Piedmont **Associations of patients** with disabilities (e.g. the Coordination Committee for Tetraplegic and Paraplegic patients of Piedmont) and neurodegenerative diseases and their families (CAAP - Coordination committee of Alzheimer Associations of Piedmont -12 local associations - the Ass. Of Parkinson friends of different provinces of the region, the Association Girotondo Onlus for SMA patients in Biella, etc.).

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

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

SCIENTIFIC SEMINARS AT NICO
21 seminars were held on Friday afternoons. For invited speakers, see the attached list. In addition, a program of 21 internal progress reports was organised (attached list included).

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2022

Laboratory name: *Adult neurogenesis*

1. LABORATORY DESCRIPTION – PERSONNEL:[1]

Principal Investigator

Principal Investigator 1

LUCA BONFANTI

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

Nationality: Italian | Gender: M

Phone: 00 39 011 6706606

Email: luca.bonfanti@unito.it

Principal Investigator 2

PAOLO PERETTO

Degree: PhD | Birthdate: 18/09/1963

Nationality: Italian | Gender: M

Phone: 00 39 011 6706605

Email: paolo.peretto@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: Lead researcher on postnatal neurogenesis in mouse models

2. FEDERICO LUZZATI

Degree: PhD | Birthdate: 20/10/1974

Nationality: Italian | Gender: M

Phone: 00 39 011 6706615

Email: federico.luzzati@unito.it

Position: Assistant professor

Role & Expertise: Lead researcher on lesion induced neurogenesis in the striatum of mammals

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

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: Lead researcher on the study of neural network involved in sexual imprinting

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. STEFANO ZUCCA

Degree: PhD Birthdate: 29/05/1988

Nationality: Italian Gender: M

Phone: 00 39 011 6706632

Email: stefano.zucca@unito.it

Position: PostDoc

Role & Expertise: two-photon and lightsheet microscopy, electrophysiology

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 *ex vivo*

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 student (35° cycle)

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 student (35° cycle)

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

10. ILARIA GHIA

Degree: Biological Sciences Birthdate: 4/01/1996

Nationality: Italian Gender: F

Phone: 00 39 011 6706632

Email: ilaria.ghia@unito.it

Position: PhD student (37° cycle – from November 2021)

Role & Expertise: Two-photon and lightsheet microscopy, histology, mouse

11. ELEONORA DALL'ORTO

Degree: Biological Sciences Birthdate: 04/07/1996

Nationality: Italian Gender: F

Phone: 00 39 011 6706632

Email: Eleonora.dallorto@unito.it

Position: PhD student (37° cycle - from November 2021)

Role & Expertise: Histology, confocal microscopy, morphometric analysis, mouse models

12. ALESSIA PATTARO

Degree: Biological Sciences Birthdate: 20/11/1997

Nationality: Italian Gender: F

Phone: 00 39 011 6706632

Email: alessia.pattaro@unito.it

Position: PhD student (38° cycle)

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

2. CURRENT GRANTS

Starting-end date	Project Title and ID	Beneficiary[2]	Funding Program/Agency	Role of the unit[3]	Overall Amount Funded	Managed by FCO/UNITO
May 2022 – April 2024	Characterization and modulation of "immature" neurons: a potentially exploitable reservoir of non-newly generated cells involved in plasticity of the adult rodent and human cerebral cortex	Luca Bonfanti	Trapezio – Compagnia di San Paolo	Coordinator	30.000	UNITO
October 2022 – September 2023	Neuroni 'immaturi' come riserva di cellule indifferenziate 'dormienti' nella corteccia cerebrale umana	Luca Bonfanti	Fondazione CRT (bandi ordinari)	Coordinator	27.000	FCO
November 2020 – October 2024	Sounds and pheromones: neural networks merging olfactory and acoustic cues in sexual imprinting	Serena Bovetti	Human Frontier Science Program	Coordinator	350.000 $/3 years	UNITO
March 2022- Nov 2024	Imprinted SCENTs: odour control of mate preference	Serena Bovetti	Trapezio – Compagnia di San Paolo	Coordinator	30000 euro	UNITO
January 2023- dec 2024	Multimodal integration of olfactory and	Serena Bovetti/Stefano Zucca	H2020 Marie Sklodowska Curie	Coordinator/ Recipient	39.000 euro	UNITO

[2] 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.

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

	acoustic cues in mouse courtship communication		Action Individual Fellowship			
January 2023- Dec 2023		Serena Bovetti	Grant for internazionalization	Coordinator	12.500 euro	UNITO

3. SCIENTIFIC ACTIVITIES IN 2022

Luca Bonfanti, Associate professor (PI)

Supervised PhD students:	Marco Ghibaudi (third year) Alessia Pattaro (first year)
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	Prof. Sebastien Couillard-Despres, University of Salzburg, Austria; Prof. Chet C. Sherwood, George Washington University, USA Prof Juan Nacher, University of Valencia, Spain; Dr. Melissa Holmes, Department of Psychology, University of Toronto, Canada
• Invited talks:[4]	- *Beyond stem cell-driven adult neurogenesis: the complex issue of "immature" neurons* (Talk at FENS Symposium, Paris, July 2022) - *Beyond stem cell-driven adult neurogenesis* Seminar at the Golgi Foundation, Abbiategrasso (MI)
• Science communication:[5]	- "*Ripensare il cervello con la plasticità cerebrale*" Online course registered for Treccani Futura (Edulia) – Treccani Encyclopedia, Rome - *Cosa c'è nella mia testa*. Libro divulgativo per ragazzi sulle Neuroscienze. Ed. Il Castoro (Luca Bonfanti, Pierdomenico Baccalario, Federico Taddia, Authors) wins the Angelo Zanibelli price 2022 (La parola che cura) as the best illustrated book - "*Tre risposte sui neuroni immaturi*" – Intervista sulla rivista BenEssere - *Plasticità e diversità* – Speaker's corners, UniVerso, Unito, Cortile del Rettorato - Participation as speaker at *Vivere per Sempre*, online streaming from NICO organized by Giovedì Scienza - Presentation on the radio of the book *L'enigma del neurone giovane* (RADAR – segnali dalla scienza, dalla cultura, dalla società)
• Editorial duties:	- Editor in chief *Frontiers in Neurogenesis* - Reviewing editor in *Frontiers in Mammal Science*

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

	- Editor of Special Issue in *Int J Mol Sci*: Neuronal and Brain Maturation Vol II (with S. Couillard-Despres) 2022 - Editor of Special Issue in *Front. Neurosci*: Insight in Neurogenesis 2023 (with Gerd Kempermann)
• others[6]	
Organizational activities and responsibilities at NICO:	Waste sorting optimization and sensitization
Speakers invited:	na
Other organizational activities:[7]	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Paolo Peretto (PI)

Supervised PhD students:	Marco Fogli (co-supervised with F. Luzzati)
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:[8]	na
Science communication:[9]	"*Suoni e odori: il linguaggio dell'amore È il cervello a scegliere il partner?*" Festival della Scienza, Genova 22 Ottobre 2022.
Editorial duties:	Associate Editor Frontiers in Neuroscience - Referee for Scientific Journals
others[10]	na
Organizational activities and responsabilities at NICO:	Representative of the personnel for safety
Speakers invited:	na
Other organizational activities[11]:	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Silvia De Marchis – Lead researcher

Supervised PhD students:	Eleonora Dallorto - Ilaria Ghia (co-supervised with S. Bovetti)
Honors, prizes, awards:	na

[6] Posters at meetings, participation in the board of scientific societies, referee for grant agencies
[7] No university appointments.
[8] Invited seminars, invited talks at meeting and symposia, invitation upon selection at meetings
[9] Public engagement
[10] Posters at meetings, participation in the board of scientific societies, referee for grant agencies
[11] No university appointments.

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; Wojciech Krezel INSERM, IGBMC, Strasbourg, France.
● Invited talks:	na
● Science communication:	-Organization of the 5th Aldo Fasolo Award for communication in neuroscience (award ceremony April 29, 2022). -Organization and participation to the Round Table entitled "Scientific Communication in the Multimedia Society" Circolo dei Lettori – CinemAmbiente June 8, 2022.
● Editorial duties:	Reviewing Editor Frontiers in Neurogenesis – Ad hoc Referee for Scientific journals
● others	**Poster presenter** ***"Novel insight into the neurodevelopmental disorder BBSOAS: Nr2f1 controls mitochondrial architecture in adult-born mouse hippocampal neurons*** FENS Forum 2022 - Paris (France), July 9-13 Representative of the PhD of Neuroscience of the University of Turin at the NENS general meeting FENS Forum 2022 - Paris (France), July 12 Member of the evaluation board of the XXXIV cycle, PhD program in Cell and Developmental Biology, University La Sapienza, Rome. May and July 2022.
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:	Coordinator of the teaching committee of the PhD program in Neuroscience. Appointed member of the Scientific Committee and of the Management Committee of the University Language Centre CLA-UniTO. Deputy scientific coordinator of UNITA Universitas Montium for UniTO.
Workshops, Schools or Conferences organized:	Comitato scientifico 94° Congresso SIBS- Biologia Sperimentale – Torino 6-9 Aprile 2022
Technology transfer achievements (patents, etc.):	na

Serena Bovetti, Associate professor

Supervised PhD students:	Ilaria Ghia (co-tutor with Silvia De Marchis)
Honors, prizes, awards:	2022: supervisor of an awarded Marie-Curie postdoctoral fellow
Outreach activities	
• International collaborations:	Dr. Paolo Giacobini, Lille, France

	Dr. Dustin Penn, Konrad Lorenz Institute, Vienna (Austria) Dr. Sylvain Gigan, ENS, Paris (France)
• Invited talks:[12]	- *Whole-brain representation of imprinted cues* (ISS, Rome, June 27, 2022) - *Whole-brain mapping of brain activity induced by imprinted cues* (Scuola Normale Superiore, Pisa, June 29, 2022)
• Science communication:[13]	- "*Suoni e odori: il linguaggio dell'amore* *È il cervello a scegliere il partner?*" Festival della Scienza, Genova 22 Ottobre 2022.
• Editorial duties:	- Associated Editor *Frontiers in Neural Circuits* - Editor of Special Issue in *Frontiers in Neural Circuits*: The neural circuitry of mating behaviors. - Reviewing editor for *Frontiers in Neural Circuits, Molecular Neurobiology, European Journal of Neuroscience*
• others[14]	*"Imaging the Mouse Developing Auditory Cortex"* GEI conference, 5-7 June 2022 Gargnano "*Whole-brain mapping of brain regions recruited by familial and unfamilial opposite-sex odors in female mice"* Human Frontier Science Program Meeting Paris August 27-30 2022
Organizational activities and responsibilities at NICO:	Responsible of the two-photon microscope Responsible of the light-sheet microscope Responsible of the BSL2 surgical room
Speakers invited:	Angelo Forli (University of California, Berkeley)
Other organizational activities:[15]	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Federico Luzzati – Lead Researcher

Supervised PhD students:	Marco Fogli (co-supervised with P.Peretto)
Honors, prizes, awards:	na
Outreach activities	
● International collaborations:	Benedikt Berninger (University of Mainz, Germany), Matteo Bergami(University of Cologne, Germany)
● Invited talks:[16]	Satellite event of the FENS Forum (2022) "*From Glia Cell Functions to Brain Dysfunctions: Understanding the Cellular Roots of Brain Disorders*", which was held on the 07th of July in Paris, France. Title of the Talk: Birth, integration and death of astrocyte-generated neurons in the adult striatum, Paris 07-07-2022. "*Decipher stem cell fate by single-cell, multiomics, and inference approaches*", University Of Turin, Molecular Biotecnology Center (MBC), 30-5/1-6 2022. Title of the talk: "*Integration of transient axonless LGE-class interneurons in the lesioned adult striatum*".

[12]Invited seminars, invited talks at meeting and symposia, invitation upon selection at meetings
[13] Public engagement
[14] Posters at meetings, participation in the board of scientific societies, referee for grant agencies
[15] No university appointments.
[16]Invited seminars, invited talks at meeting and symposia, invitation upon selection at meetings

• Science communication:[17]	- C'era una volta un neurone (Once upon a time there was a neuron), Conference show to explain the basic functions of the nervous system. Held 19/12/2022 at the opening ceremony of the event "Bambine e Bambini all'Università" organized by the University of Turin
• Editorial duties:	na
• others[18]	Reviewer for international journals
Organizational activities and responsibilities at NICO:	na
Speakers invited:	
Other organizational activities:[19]	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Sara Bonzano - PostDoc

Supervised PhD students:	Eleonora Dallorto
Honors, prizes, awards:	na
Outreach activities	
1. International collaborations:	R. Berckervordersandforth and D.C. Lie (FAU - Erlangen, Germany); M. Studer (iBV, UCA, CNRS, INSERM - Nice, France)
2. Invited talks:[1]	Jacques Monod Conference 2022: Genetics, environment, signalling & synaptic plasticity in developmental brain disorders: from bench to bedside. Title: "New insight into the neurodevelopmental disorder BBSOAS: the role of Nr2f1 on mitochondrial architecture in adult-born mouse hippocampal neurons". Roscoff (France); April 11-15, 2022
3. Science communication:[2]	Participation to "INTOtheBRAIN connections you don't expect" organized by NICO on the occasion of the international initiative "UNIGHT - United citizens for research". Role: Guide to confocal microscopy and its use in research.
4. Editorial duties:	Referee for: Metabolic Brain Disease Journal of Experimental Neuroscience
5. others[3]	NICO NeuroWebinar: "A Pilot Investigation of Nr2f1 expression and functions during Experience-dependent Neuroplasticity in the Adult Mouse Dentate Gyrus" ; May 10, 2022
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:[4]	na
Workshops, Schools or Conferences organized:	na

[17] Public engagement

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

[19] No university appointments.

Technology transfer achievements (patents, etc.):	na

Stefano Zucca, Post-doctoral fellow

Supervised PhD students:	na
Honors, prizes, awards:	- *Best Oral Presentation*: GEI-Italian Society of Development and Cell Biology (GEI-SIBSC), 2022 - *Project Support Grant* - British Society for Neuroendocrinology (5780£) - *H2020 Marie Sklodowska Curie Action Individual Fellowship* – Awarded & Seal of Excellence
Outreach activities	
• International collaborations:	- Dr. Paolo Giacobini, Lille, (France) - Dr. Dustin Penn, Konrad Lorenz Institute, Vienna (Austria) - Dr. Sylvain Gigan, ENS, Paris (France)
• Invited talks:	- *"Academic Stressors: taking care of our mental health"*. FEBS May 2022, Advanced Course, Crete, Greece - *"Academic Stressors: taking care of our mental health"*. Pittsburg University, Pittsburg, USA. September 2022 - *"Mental health in academia: stressors among underrepresented groups"*. MSCA ASTROTECH Conference, Milan, Italy. December 2022 - *"Midbrain representation of looming stimuli"*. University of Sussex, Sussex, United Kingdom - *"Whole brain representation of imprinted odours"*. GEI-Italian Society of Development and Cell Biology (GEI-SIBSC), June 2022.
• Science communication:	
• Editorial duties:	- Editor of Special Issue in *Frontiers in Neural Circuits*: The neural circuitry of mating behaviors.
• Others	*Poster presenter"Whole brain mapping of brain regions recruited by imprinted and unfamiliar opposite sex odours in female mice".* Human Frontier Science Program Meeting Paris August 27-30 2022
Organizational activities and responsibilities at NICO:	
Speakers invited:	- Letizia Mariotti (University of Padova) - Bianca Silva (Humanitas Research Hospital)
Other organizational activities:	Responsible for the in vivo electrophysiology setup
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Chiara La Rosa, Post-doctoral fellow

Supervised PhD students:	na
Honors, prizes, awards:	- Awarded of a Post-Doctoral Fellowship from Fondazione

	Veronesi. Project: *Effects of noise pollution on the mouse developing auditory system.* - Awarded of a FENS-IBRO/PERC Travel Grant 2022 to attend the FENS Forum 2022, Paris, France, 9-13/07/2022.
Outreach activities	
• International collaborations:	Dr. Dustin Penn, Konrad Lorenz Institute, Vienna (Austria) Dr. Sylvain Gigan, ENS, Paris (France)
• Invited talks:	na
• Science communication:	- *La Ricerca si racconta* Fondazione Umberto Veronesi, Liceo Scientifico Federico II, Altamura, Bari, 25/11/2022. - *Ricercatori in Classe* Fondazione Umberto Veronesi, Liceo Scientifico Filippo Juvarra, Venaria, Torino, 21 & 27/04/2022.
• Editorial duties:	Reviewer for *Scientific Reports, Brain Sciences*.
• others	C. La Rosa, S. Zucca, P. Peretto, S. Bovetti - *Imaging the mouse developing auditory cortex.* FENS Forum 2022, Paris, France, 9-13/07/2022.
Organizational activities and responsibilities at NICO:	Person in charge for the organization of the office space for PhD students and postdocs.
Speakers invited:	na
Other organizational activities:	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Ilaria Ghia, PhD Student

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	na
• Invited talks:	na
• Science communication:	*"6th Swiss Science Filmmaking Marathon"* Zurich September 15-17 2022
• Editorial duties:	
• others	*Poster: "Validation of a method to specifically label dopaminergic cells in the mouse olfactory bulb" SINS National Meeting of PhD Students in Neuroscience 2022. Brescia June 11 2022*
Organizational activities and responsibilities at NICO:	Member of the NICO Green Committee
Speakers invited:	na
Other organizational activities:	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Eleonora Dallorto, PhD student

Supervised PhD students:	na
Honors, prizes, awards:	"Young investigator award" 94° Congresso SIBS 2022
Outreach activities	
• International collaborations:	Michèle Studer (iBV, UCA, CNRS, INSERM - Nice, France)
• Invited talks:	na
• Science communication:	*"6th Swiss Science Filmmaking Marathon"* Zurich September 15-17 2022
• Editorial duties:	na
• others	Posters: - BRAYN Conference 2022 - Rome (Italy), 28-30 September 2022 "*Nr2f1 haploinsufficiency affects immature granule neurons morphology and leads to an altered activation of neuronal ensembles within the adult mouse hippocampus".* - FENS Forum 2022 - Paris (France), July 9-13 2022 "*Nr2f1 haploinsufficiency alters the morphology of adult-born neurons in the hippocampus of a mouse model of the neurodevelopmental disorder BBSOAS*" - SINS National Meeting of PhD Students in Neuroscience 2022 - Brescia (Italy), June 11 2022 "*Morphological characterization of adult-born hippocampal neurons in a mouse model of the neurodevelopmental disorder BBSOAS*". - Jacques Monod Conférence: Genetics, environment, signaling, & synaptic plasticity in developmental brain disorders: from bench to bedside - Roscoff (France), April 11-15 2022 "*Morphological characterization of adult-born hippocampal neurons in a mouse model of the neurodevelopmental disorder BBSOAS*". - 94th National Congress of the Italian Society of Experimental Biology (SIBS) 2022 - Turin (Italy), April 06-09 2022 "*Morphological characterization of adult-born hippocampal neurons in a mouse model of the neurodevelopmental disorder BBSOAS*" - NICO NeuroWebinar: "*A study on the effects of Nr2f1 haploinsufficiency in the postnatal hippocampus*", October 21st 2022
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:	-PhD student representative of the PhD program in Neuroscience -Member of the Internationalization committee (Department of Life Sciences and Systems Biology - DBIOS)
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

ALL LAB MEMBERS

Activities:[20]	

[20] 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 2022[21]

a. Summary (500 characters)

Different aspects of postnatal/adult brain plasticity in healthy and pathological conditions were addressed:

i) the role of olfactory and acoustic cues in the shaping of neural circuits for sexual imprinting
ii) the existence of *"immature"* neurons in adult mammals, from small-brained to large-brained species
iii) the effect of Nr2F1 haploinsufficiency on adult neurogenesis (AN)
iv) the mechanisms and dynamics of lesion-induced acquisition of a neurogenic competence in striatal astrocytes.

b. Background and rationale (3000 characters)

The brain's ability to adapt its organization and function is driven by environmental cues and achieved through complex cellular and molecular mechanisms that primarily occur during the postnatal critical periods. Plasticity processes continue to some extent in the mature brain involving the generation of new neurons in specific sites (i.e. adult neurogenic niches) and/or the persistence of neurons in an immature state. However, several questions remain open regarding the early/postnatal sensory-driven shaping of cerebral circuits, as well as the existence and role of newly generated and/or immature neurons in various mammals, including humans, under both physiological and pathological conditions.

In this complex picture, some pivotal questions are:
1) How and when the olfactory and acoustic cues shape neural brain circuits underlying reproduction? It is well accepted that early exposure to such stimuli heavily influences the reproductive behavior of females. However, no data are still available about when this process occurs during postnatal development, which circuits integrate these cues, and the relative importance of the presence of father in the nest to accomplish this mechanism.

2) How different types of plasticity (AN versus "immature" neurons) are phylogenetically distributed among mammals? How widespread are immature neurons in the mammalian brain? These questions arise by recent studies revealing conflicting results and interpretations on the existence and function of AN in the human brain, and unveiling new/alternative types of structural plasticity (i.e., immature neurons).

3) how is regulated the fate of adult neural stem cells (NSCs) and function of adult born neurons in physiological or pathological conditions?

The rationale of the research carried out in 2022 can be summarized as follows:

1. In many animal species, the ability to establish memories of relatives during infancy is fundamental for several vital behaviours. One among many is sexual imprinting, a process of instinctive learning that happens early during development, when individuals acquire memories of the odours, vocalizations, and other characteristics of their parents (or siblings), and then utilize this information to select their mates as adults. During this year we analyzed the brain areas activated by exposure to familiar (father) and unfamiliar

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

(unrelated male) odors to assess whether previous exposure to father during development could shape the recruitment of specific brain pathways.

2. 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.

3.1. Mutations in the NR2F1 gene are responsible for causing BBSOAS (ORPHA:401777), a rare autosomal-dominant disorder that is characterized by various clinical features, including mild-to-severe intellectual disability and autism spectrum disorder. However, the underlying cellular and molecular mechanisms of this disorder are still largely unknown. Our previous research has shown that Nr2f1 is highly expressed in the adult mouse hippocampal dentate gyrus (DG), where it plays a crucial role in directing neural progenitors towards a neuronal fate and shaping mitochondria in adult-born neurons.

3.2 We demonstrated that the striatal parenchyma can be permissive for neurogenic activation of local astrocytes, however the mechanisms and dynamics of progenitors activation and expansion outside the canonical niches remains unclear. During this year we have tackled this question with multiple approaches including lineage tracing, spatial and clonal analyses. In parallel, we have extended a previous analysis on the fate of the newborn neurons trough single cell RNA seq.

c. Objectives (1000 characters)

i) Examine the brain regions involved in the formation of sexual imprinting

ii) Investigate whether subcortical "immature" neurons are heterogeneous across different mammalian species and potentially more prevalent in large-brained species. Additionally, the study aimed to begin investigating cortical immature neurons in human fetal brains.

iii) Explore the impact of Nr2f1 haploinsufficiency on adult neurogenesis

iv) Determine the dynamics and mechanisms of striatal astrocytes neurogenic potential and analyze the identity and integration capacity of their neuronal progeny.

d. Results (4000 characters)

i) *Neural network involved in sexual imprinting.* C57BL6J females were raised in the presence or absence of their father up to weaning. At 3 months of ages, females were acutely exposed either to the urine of their own father or to urine of a sexually experienced male of a different strain (unfamiliar). Brains were then processed and the activated brain areas were mapped by looking at cFOS expression. In females raised with father, both stimuli (i.e. father or unrelated male urine) significantly increased neuronal activity compared to the control group, with unfamiliar cues recruiting a larger number of areas compared to familiar ones. Among the recruited areas, 14 of them overlapped between the two conditions. These include key brain regions involved in olfactory processing, valence and social recognition, mating behaviours and hormonal regulation. Importantly, we identified a subset of hypothalamic nuclei activated by unfamiliar odours only. These data provide a comprehensive analysis of brain recruitment by opposite-sex cues and allow to identify target regions where the discrimination between imprinted and unfamiliar odours might occur.

ii) *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, "dormant" 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.

iii) *Nr2f1 haploinsufficiency*. We analysed constitutive Nr2f1 heterozygous mice (Nr2f1-HET), a recently validated mouse model of BBSOAS, and focused on the dentate gyrus (DG). Our results indicate that Nr2f1 haploinsufficiency does not alter the total number of DCX+ neuroblasts/immature neurons in the adult DG. However, these cells in Nr2f1-HET mice exhibit atypical neuronal morphologies that are often associated with pathological conditions and aberrant hippocampal circuitry activation. Further analysis revealed an increased activation of mature granule neurons in the DG of Nr2f1 heterozygous mice, as evidenced by increased expression of immediate early genes (e.g., Npas4, c-fos). Moreover, patch-clamp recordings of DG granule cells from hippocampal slices suggest reduced inhibition on granule cells in HET mice. Ongoing immunofluorescence analyses using pre- and post-synaptic markers of inhibitory and excitatory synapses are further supporting a possible excitatory/inhibitory imbalances in the DG of HET mice.

iv) *Lesion induced striatal neurogenesis*. Numerous neurogenic foci can be observed in the striatum for at least six months following injury. Through clonal analyses in confetti mice, we have demonstrated that these foci originate from the clonal expansion of individual astrocytes. Lineage tracing, BrdU analyses, and mathematical modeling have indicated that striatal astrocytes undergo sporadic activation, which establishes transient foci that are continually turning over. This spatiotemporal dynamic closely resembles that observed in canonical neurogenic niches, indicating that, contrary to previous belief, neurogenic competence is widespread among striatal astrocytes, and the striatal parenchyma is largely permissive for its expression. In parallel, we have extended the single-cell RNAseq analysis of the neuronal progeny and definitively demonstrated that these cells are not committed to striatal cell types but correspond to a neuron type that exists in the striatum only during postnatal development.

e. Advancement in the field (1000 characters)

i) *Neural network involved in sexual imprinting*: through Light Sheet Microscopy we identified several, previously undescribed, female brain regions differentially activated by exposure to male olfactory cues.

ii) *Immature neurons:* 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.

iii) *Nr2f1 haploinsufficiency*: new insights on the mouse BBSOAS model revealing alteration in the excitatory/inhibitory balances in the DG of adult mice.

iv) *Lesion induced striatal neurogenesis*: We have demonstrated that the brain parenchyma can be remarkably permissive for neural stem cell activity. Furthermore, we have shown

that lesion-induced striatal neurogenesis may represent a new form of compensatory plasticity that could be useful for the reorganization of damaged circuits.

f. Publications[22]

1. Ghibaudi M, Bonfanti L. 2022 How Widespread are the "Young" Neurons of the Mammalian Brain? Front. Neurosci. 6;16:918616.

2. Bonfanti L, Couillard-Després S. 2022 Neuronal and Brain Maturation. Int. J. Mol. Sci. 23(8):4400.

3. Boda E, Lorenzati M, Parolisi R, Harding B, Pallavicini G, Bonfanti L, Moccia A, Bielas S, Di Cunto F, Buffo A. Molecular and Functional Heterogeneity in Dorsal and Ventral Oligodendrocyte Progenitor Cells of the Mouse Forebrain in Response to DNA Damage. Nat. Commun. 2022 13(1):2331.

4. Malloul H, Bonzano S, Bennis M, De Marchis S, Ba-M'hamed S. 2022 Chronic Thinner Inhalation Alters Olfactory Behaviors in Adult Mice. Behav. Brain Res. 6;23(11):6351.

5. Fornasari BE, Zen F, Nato G, Fogli M, Luzzati F, Ronchi G, Raimondo S, Gambarotta G. 2022 Blood Vessels: The Pathway Used by Schwann Cells to Colonize Nerve Conduits. Int J Mol Sci. 23(4):2254.

6. Oberto A, Bertocchi I, Longo A, Bonzano S, Paterlini S, Meda C, Della Torre S, Palanza P, Maggi A, Eva C. 2022 Hypothalamic NPY-Y1R Interacts with Gonadal Hormones in Protecting Female Mice against Obesity and Neuroinflammation. Int. J. Mol. Sci. 6;23(11):6351.

5. Future directions and objectives for next years

a. Summary (up to 2000 characters):

Our more recent studies have been focused on exploring new and alternative angles of neural plasticity mechanisms underlying brain function/development: the molecular control of neuronal-glial switch in neurogenic sites, the activation of quiescent (neurogenic) astroglial progenitors in the lesioned striatum, the comparative approach to phylogenetic variation of non-newly generated "immature" neuronal populations in mammals, the role of sensory cues in shaping the organization and function of brain circuits critical for survival. In the next year, we will focus on the same topics through an in depth analysis of the molecular/cellular mechanisms regulating adult NSC and immature neuron function in both physiological and pathological conditions and a further characterization of the immature neuron "reservoirs" in widely different mammals (including humans) and brain regions. Moreover, we will continue to investigate the organization and function of neural circuits integrating olfactory and acoustic cues responsible for sexual imprinting in female mice (according to HFSP founded research project).

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

Studies performed during the last 30 years on unravelling mechanisms driving adult brain organization and function, have revealed that brain plasticity plays a key role in shaping neural circuits critical for survival. From one side it has been clearly established the brain organizational importance of several external/environmental (e.g., olfactory, visual, acoustic stimuli) and internal

[22] DO NOT include papers in press or submitted.

cues (e.g., hormones) acting during the peri- and post-natal critical periods. From the other side, the discovery of adult neurogenesis in mammals, as a further mechanism of adult neural plasticity, has opened interesting perspectives to understand brain function both in physiological and pathological conditions. Nevertheless, several unanswered questions still remain to translate these knowledges into general basic rules of neural organization/function and, possibly, to develop therapeutical strategies.
It is known that early/post-natal exposure to paternal acoustic and olfactory stimuli is critical to shape mate choice in female mice, nevertheless the neural bases of this mechanism are largely unknow. Unravelling this process will increase our knowledge about the shaping of circuits allowing multisensory integration. The research in the field of adult neurogenesis, although has demonstrated in rodents its involvement in terrific cognitive functions (e.g., memory and learning), it is also showing that this mechanism can be significantly heterogenous among mammals, and limited to the postnatal period in humans. Nevertheless, 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 related or not to AN that potentially influence, complement or compensate the role of AN in the human brain. This new vision rises new problems, opportunities and questions, such as:

A) how different types of plasticity (AN versus "immature" neurons) are phylogenetically distributed among mammals and in different brain regions?
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
B) 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 the identification of neural circuits responsible for sexual imprinting in female mice (according to the founded HFSP research project). It is known that female mice use olfactory and acoustic cues from parents to learn and form memories of conspecifics and close kin, which enable 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) how is regulated the fate of adult neural stem cells (NSCs) and function of adult born neurons 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).

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)

- *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 human fetal brains* Based on an agreement with the Hospital S. Anna in Turin, we are collecting human fetal brains at different gestational stages that will be processed in order to study the development of immature neurons in the cerebral cortex layer II and establish their total amount shortly before birth. This will give an estimation of the immature neuron reservoir in humans.

-*Neural network involved in sexual imprinting.* Based on the preliminary results obtained during the first year of the project (2020-2021) we aim to run behavioral assays and subsequent whole brain immunostaining and imaging in wild mice to identify recruited areas during the recall of imprinted memories. This part of the project will be performed in collaboration with Dr. Dustin Penn (University of Vienna), partner of the project and leader in the study of reproductive behavior in wild mice. Moreover, we aim to finalize the final design and approach of the imaging technology addresses to allow in vivo imaging of neural circuits in freely-moving animals (living in the wild) during mating behavior. This part of the project will be performed in collaboration with Dr. Sylvain Gigan (Sorbonne University, Paris, France) third partner of the project and word expert in the development of advanced optical tools.

-*Hippocampal neurogenesis as a model to study BBSOAS* and the causative mechanisms and pathogenesis of intellectual disability. Stemming from our recent data on Nr2f1 function we aim to further characterize the mitochondrial phenotype in hippocampal newborn neurons of Nr2f1 mutant mouse models, including models carrying Nr2f1 human mutation, exploiting electron microscopy, biochemical and functional analysis as well as electrophysiology by *in vivo*/*ex vivo* approaches.

-*Mechanisms and role of astrocyte neurogenic activation.* The mechanisms and role of astrocyte neurogenic activation have been studied extensively. Previous research has demonstrated that the transcription factor SOX2 is sufficient to induce neurogenic activation in striatal astrocytes and is also necessary for astrocyte reactivity after injury. Our preliminary results indicate that deleting the SOX2 in approximately half of the striatal astrocytes completely abrogates the neurogenic response to QA lesion. By varying the time and efficiency of deletion, we aim to differentiate between cell-autonomous and non-cell-autonomous roles of SOX2 at different stages of striatal astrocyte neurogenic activation. These findings will guide RNAseq analysis at the tissue and single cell levels, which is aimed at identifying intrinsic and extrinsic factors that regulate the striatal neurogenic niche. Additionally, we will conduct a detailed analysis of the phenotype of the transient population of newborn neurons by examining their morphology, connectivity, and transcriptomic profile in various models of striatal neurogenesis.

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

In our research group, we address different aspects of brain structural plasticity, ranging from classic adult neurogenesis to "immature" neurons, and including progenitor specification, hormone-linked behavior, lesion-induced repair, and the reservoir of "young" neurons. We employ a combination of

basic and innovative technical approaches to study different types of plasticity occurring in various brain regions of different mammalian species, from mice to humans, at the molecular, cellular, and functional levels. We believe that this comparative approach, from molecule to behavior, could broaden our understanding of brain plasticity and help us translate research data from animal models to humans accurately.
One crucial point we aim to address is the identification of mechanisms underlying the neuronal-glial switch in both neurogenic and non-neurogenic sites to modulate endogenous progenitors. We also investigate "immature" neurons and use imaging technology in wild-living mice, which are novel topics currently addressed by only a few laboratories worldwide.
Furthermore, we are searching for a promising neuronal population that is abundantly present in large-brained mammals with reduced rates of adult neurogenesis, with particular reference to humans. We believe that this approach, in addition to providing new insights into basic neurobiology, will help overcome the current bottleneck of the "classic" adult neurogenesis vision, which is the constitutive, continuous genesis of new neurons in rodents. Instead, we explore the less-traveled roads mentioned above.

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

Our research group is developing various innovative technologies to achieve the goals of our projects. We employ in vivo two-photon microscopy with fluorescent cell activity reporters (GCaMP) in head-restrained anesthetized and awake mice to study the functional role of neurons in diverse brain circuits (e.g., olfactory bulb and cerebral cortex) after exposure to salient sensory cues such as olfactory and acoustic stimuli. We also use two-photon imaging to investigate mitochondrial dynamics in neurogenic regions.
In collaboration with Dr. S. Gigan from the Laboratoire Kastler-Brossel Sorbonne Université in Paris and Dr. D. Penn from the Konrad Lorenz Institute of Ethology in Vienna, our HFSP research project aims to develop a high-throughput imaging technology based on multimodal optical fibers integrated in a wire-free head-mounted device. This approach will allow us to record the functional activity from multiple brain regions with single-unit resolution, low invasiveness, and in freely-moving animals. We plan to use this technique to simultaneously image the brain regions involved in mating behavior, with a primary focus on olfactory-related areas.
We have developed a customized approach to standardize and automate the production of serial section reconstructions through hierarchical imaging at the confocal microscope. This enables us to obtain 3D high-resolution reconstructions of large volumes. We use block face imaging of the specimen during sectioning as a reference for non-linear registration of the confocally acquired volumes to their original position in the intact brain. We have already used a preliminary version of this method to reconstruct the distribution of GnRH+ cells in the entire brain, the composition of neurogenic niches in the lesioned striatum, the morphology of newly generated neurons, and the distribution of their afferents. This technique may be useful in studying immature neuron populations and their modulation."

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2022

Laboratory name: Physiopathology of neural stem cells

1. LABORATORY DESCRIPTION – PERSONNEL:[23]

Principal Investigator
Annalisa Buffo, Associate Professor of Physiology, PhD, 25-12-1967, +39 0116706614, annalisa.buffo@unito.it

Personnel
Enrica Boda, Associate Professor of Anatomy, PhD, 08-05-1981, +39 0116706615, enrica.boda@unito.it. Lead responsible of research on oligodendroglial physiopathology

Roberta Parolisi, Senior PostDoc, PhD, 23-01-1985, +390116706632, roberta.parolisi@unito.it. Responsible of EM and 3D lightsheet microscopy investigations, expert in myelin ultrastructure

Valentina Cerrato, Senior PostDoc, PhD, 21-07-1988, +390116706615, valentina.cerrato@unito.it. Responsible of research on astrocyte heterogeneity & cerebellar development; expert in clonal/single cell analyses

Giulia Nato, Senior PostDoc, PhD, 08-05-1986, +390116706632, giulia.nato@unito.it. Responsible of research on astrocyte neurogenic activation and reactivity

Martina Lorenzati, Junior PostDoc, PhD, 30-10-1992, +390116706632, martina.lorenzati@unito.it, Expert in oligodendroglia biology and derivation of neural cells from hPSCs

Gabriela Berenice Gómez-González, Junior PostDoc, PhD, 06-04-1987, +390116706632, gabrielaberenice.gomezgonzalez@unito.it. Functional studies of human neurons implanted in a rat model of Huntington's disease

Marta Ribodino, PhD candidate, MSc in Biotechnology, 01-06-1996, +390116706632, marta.ribodino@unito.it. Derivation of glia from hPSCs, cell therapy approaches in Huntington Disease's models

Maryam Khastkhodaei Ardakani, PhD candidate, MSc in Anatomical Sciences, 30-06-1991, +390116706632, maryam.khastkhodaeiardakani@unito.it. Pharmacological approaches to promote recovery in microcephaly models

Martino Bonato, PhD candidate, Master in Molecular Biotechnology, 09-03-1997, +390116706632, martino.bonato@edu.unito.it. Study of particulate matter effects in Multiple Sclerosis models

Ersilia Nicorvo, Junior Research fellow, Master in Neuroscience, 31-05-1995, +390116706632, ersilia.nicorvo@unito.it. Support in human PSC-derived cell cultures

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

2. CURRENT GRANTS

Starting-end date	Project Title and ID	Beneficiary[24]	Funding Program/Agency	Role of the unit[25]	Overall Amount Funded	Managed by FCO/UNITO
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 €	UNITO
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 €	UNITO
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	Cassa di Risparmio di Torino (CRT) Foundation	Coordinator (PI)	20,000 €	UNITO
01/10/2021-31/03/2023	Air pollution and Multiple Sclerosis: effects of the exposure to particulate matter (PM) on neuroinflammation and myelin repair (ID 2021.0657)	Enrica Boda	Cassa di Risparmio di Torino (CRT) Foundation	Coordinator (PI)	20,000 €	FCO
01/04/2022-31/03/2023	Air pollution and Multiple Sclerosis: role of particulate matter (PM) exposure and associated extracellular vesicle trafficking in	Roberta Parolisi	Umberto Veronesi Foundation	Fellowship beneficiary	30,000 €	na

[24] 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.

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

	neuroinflammation and demyelination (ID: 4674)					
1/12/2021-31/07/2023	SPACER - a single cell SPAtiotemporal transcriptomic atlas to unveil CERebellar development and function in mouse	Annalisa Buffo	EASI Genomics	Coordinator (PI)	Costs covered for the on site execution of the proposed experiment (about 50,000 €)	na
1/1/2022-30/6/2023	SPACER - Un atlante di trascrittomica spaziale in singola cellula per studiare lo sviluppo e le funzioni del cervelletto	Annalisa Buffo/Valentina Cerrato	Banca d'Italia	Coordinator (PI)	25,000 €	FCO

3. SCIENTIFIC ACTIVITIES IN 2022

Annalisa Buffo, PI

Supervised PhD students:	Maryam Khastkhodaei (co-supervised with E. Boda) Marta Ribodino
Honors, prizes, awards:	-
Outreach activities	
• International collaborations:	NSC-Reconstruct network (main collaborators: E Cattaneo, University of Milano, M Parmar, University of Lund; A Bosio, Miltenyi Biotec, Koln; M Gotz, University of Muenchen). See also specific collaborations of lab members below.
• Invited talks:[26]	'Striatal neuron transplantation for cell replacement in Huntington's Disease: prospects and challenges' Webinar Italy-Japan approaches to neural regeneration. June, 8 2022 https://www.nico.ottolenghi.unito.it/Agenda/Italy-Japan-approaches-to-neural-regeneration 'Reconstructing brain circuits: Lessons from endogenous neurogenic responses and human striatal grafts' Invited seminar, BMC, LMU, Munich, September 1 2022
• Science communication:[27]	UNISTEM Day Torino 2022 – organizer and participant - (UniStem Day XIV - Diretta streaming su Unito media | Università di Torino)

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

[27] Public engagement

	BRAIN AWARENESS WEEK (La Settimana del Cervello) – organizer and participant - (Dal 14 al 18 marzo torna la Settimana del Cervello / News / Home - Nico (unito.it)) "Vivere per sempre" Giovedì Scienza racconta la ricerca al NICO -organizer and participant -(Vivere per sempre: GiovedìScienza racconta la ricerca al NICO (unito.it) NICO UNIGHT INTOtheBRAIN -organizer and participant - connessioni che non ti aspetti tra inquinamento, vivere green e cervello (UNIGHT il 30 settembre torna la Notte europea dei ricercatori (unito.it)) UNITO UNIGHT INTOtheBRAIN -organizer and participant- connessioni che non ti aspetti tra inquinamento, vivere green e cervello, Caffè Scientifico alla Casa del Quartiere (Notte dei ricercatori, vivere green e gli effetti benefici sul cervello- Corriere.it)
• Editorial duties:	Ad hoc reviewer for the following journals: Science Advances, Nature Communications, Plos Biology, Glia, Clinical and Translational Medicine, Cerebellum, Frontiers in Neuroanatomy, Frontiers in Neuroscience, Sci Reports. Review Editor for Frontiers in Neuroanatomy Review Editor for Frontiers in Cellular Neuroscience – Section Non-neuronal cells Review Editor for Frontiers in Neurogenesis
• others[28]	Grant reviewer for the following funding agencies: MICINN-ANEP, Spanish Ministry of research and innovation FISM, Italian Foundation for Multiple Sclerosis Università Italo Francese, VINCI program Reviewer and Jury component for the national award 'Premio Giovedì Scienza', Centro Scienza, Torino Membership in Scientific Societies: Federation of the European Neuroscience Societies (FENS) Italian Society of Neuroscience (SINS) Member of the task force for Ataxia (Society for Research on the Cerebellum and Ataxias) International Society for Stem cell Research (ISSCR) Society For Research On The Cerebellum And Ataxias (SRCA) Attended meetings: NECTAR 2022 Conference, 24-26 October 2022 ISSCR 2022 meeting 14-16 JUNE, 2022 (virtual participation) Annual meeting of the NSC-Reconstruct Consortium, 5-7 April 2022, Bellagio, Italy
Organizational activities and responsibilities at NICO:	- Deputy Director of NICO - CEO of S&P Brain - Responsible of BLS2 labs at NICO - Member of the "Public Engagement Committee" of the NICO
Speakers invited:	Giulia Ramazzotti, Università di Bologna (21/01/22);

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

	Alessandro Bertero, MBC, Univ. Torino (18/02/22) Dilek Colak (Cornell University) (18/03/22) Maria Concetta Miniaci (Univ. Napoli) (07/12/22)
Other organizational activities:[29]	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Enrica Boda, Associate Professor[30]

Supervised PhD students:	Maryam Khastkhodaei (co-supervised with A Buffo); Martino Bonato
Honors, prizes, awards:	-
Outreach activities	
● International collaborations:	Prof. Stephanie Bielas and Dr. Amanda Moccia (Dept. of Human Genetics, University of Michigan, Ann Arbor, MI, USA)
● Invited talks:	"Air pollution and Multiple Sclerosis: role of particulate matter (PM) exposure and associated extracellular vesicle trafficking in neuroinflammation and demyelination". Annual Scientific Congress of the Italian Multiple Sclerosis Association. 25 May 2022, Rome, Italy
● Science communication:	- UNITO UNIGHT INTOtheBRAIN connessioni che non ti aspetti tra inquinamento, vivere green e cervello, Caffè Scientifico alla Casa del Quartiere ((Notte dei ricercatori, vivere green e gli effetti benefici sul cervello- Corriere.it) - 20/09/2022 Interview live on facebook channel PPHC - Policy & Procurement in HealthCare -Connessioni che non ti aspetti tra inquinamento e cervello / Agenda / Home - Nico (unito.it) - 10/05/2022 Interview Radio 24 Obiettivo Salute (https://www.radio24.ilsole24ore.com/programmi/obiettivo-salute/puntata/trasmissione-10-maggio-2022-121642-AEM8wfXB) - 06/05/2022 Interview GRP TV - Obtained IBRO fundings for Science Dissemination (5,000 EUR) and organized the NICO Virtual Tour
● 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) Guest Editor for the Research Topic "Methods for Neuroscience Research: from Molecular to Cellular Investigations", Front Mol Neurosci (https://www.frontiersin.org/research-topics/45851/methods-for-neuroscience-research-from-molecular-to-cellular-investigation)

[29] No university appointments.

[30] Please duplicate the module for the various lab members

	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 (approx. 14 review/year) for: Nat Commun, Glia, Progress in Neurobiology, Advanced Science, Front Neurosci, Sci Rep, Mech Ageing Dev, Mol Neurobiol, Eur J Neurosci, Journal of Alzheimer's Disease (JAD), SpringerNature NeuroMethods book series, etc.
● others	Grant Reviewer for: Italian Foundation Multiple Sclerosis (FISM) (2021-2022) National Science Center – Poland (2022) Giovedì Scienza Award (2022) Member of the "Conseil de Perfectionnement" of the Master Program in Neuroscience, Université de Paris, France (https://master-neuroscience-paris.fr/) Phd Committee member for: Dr. Aïda Padilla-Ferrer - Role of the oligodendroglial ADAM10 in re/myelination of the central nervous system. Doctoral School "Medicine, Toxicology, Chemistry, Imaging", Université Paris Descartes, France PhD external reviewer for: Dr. Stefania Zorzin – The role of meninges and meningeal neural stem cells in health and disease. PhD in Neuroscience, Psychological and Psychiatric Sciences, and Movement Sciences, University of Verona, Italy Membership in Scientific Societies: Federation of the European Neuroscience Societies (FENS) European Society of Neurochemistry (ESN) Italian Society of Neuroscience (SINS) Italian Group for the Study of Neuromorphology (GISN) Italian Society of NeuroImmunology (AINI) BraYn (Brainstorming Research Assembly for Young Neuroscientists) Association Posters at meetings: **Boda E**, Lorenzati M, Parolisi R, Harding B, Pallavicini G, Bonfanti L, Moccia A, Bielas S, Di Cunto F, Buffo A (2022) *Molecular and functional heterogeneity in dorsal and ventral oligodendrocyte progenitor cells of the mouse forebrain in response to DNA damage.* FENS Meeting, Paris, July 2022 Bonato M, Montarolo F, Parolisi R, Pandino C, Bertolotto A, Buffo A, **Boda E**. *Selective behavioral alterations after acute particulate matter exposure in a pre-symptomatic Multiple Sclerosis mouse model.* SINS PhD meeting- Italian Society for Neuroscience - June 11th, 2022, Brescia, Italy

	Bonato M, Montarolo F, Parolisi R, Pandino C, Bertolotto A, Buffo A, **Boda E**. *Selective behavioral alterations after acute particulate matter exposure in a pre-symptomatic Multiple Sclerosis mouse model.* 5th Brainstorming Research Assembly for Young Neuroscientists (BraYn congress) 2022 (28-30 September, Rome) Khastkhodaei Ardakani M, Astigiano C, Ferrini F, La Rosa C, Schellino R, Boido M, Bovetti S, Buffo A, **Boda E**. *Rescuing neural cell survival and maturation in a primary autosomal recessive microcephaly-17 (MCPH17) mouse model: effects of the postnatal N-acetyl cysteine treatment.* SINS PhD meeting-Italian Society for Neuroscience - June 11th, 2022, Brescia, Italy Khastkhodaei Ardakani M, Astigiano C, Ferrini F, La Rosa C, Schellino R, Boido M, Bovetti S, Buffo A, **Boda E**. *Rescuing neural cell survival and maturation in a primary autosomal recessive microcephaly.* 5th Brainstorming Research Assembly for Young Neuroscientists (BraYn congress) 2022 (28-30 September, Rome)
Organizational activities and responsibilities at NICO:	- Responsible for the Histology Lab; - Organizer of the Progress Report seminar series; - Member of the Public Engagement Committee; - Member of the Green Policy Committee; - Organizer of the annual Plogging walk in the frame of M'Illumino di Meno Day
Speakers invited:	-
Other organizational activities:	-
Workshops, Schools or Conferences organized:	Member of the Organizing and Scientific Committee of the BraYn (Brainstorming Research Assembly for Young Neuroscientists*)* Conference, 28-30 September 2022, Rome, Italy. https://www.braynconference.com/
Technology transfer achievements (patents, etc.):	-

Roberta Parolisi, senior PostDoc

Supervised PhD students:	na
Honors, prizes, awards:	Fellowship granted by Fondazione Umberto Veronesi, Milan, Italy, for a 12-month. Title of the project: "Air pollution and Multiple Sclerosis: role of particulate matter (PM) exposure and associated extracellular vesicle trafficking in neuroinflammation and demyelination".
Outreach activities	
● International collaborations:	NSC-Reconstruct Partners: Elena Cattaneo, Coordinator (Università di Milano), Malin Parmar (University of Lund), Oliver Bruestle (UniversitaetsKlinikum Bonn), Ernest Arenas (Karolinska Institutet), Roger Barker (University of Cambridge), Agnete Kirkeby (Kobenhavns Universitet), Meng Li (Cardiff University), Magdalena Goetz (Helmholtz Zentrum Muenchen), Pierre Vanderhaeghen (VIB-KULeuven Center) , Andreas Bosio (Miltenyi Biotec GMBH), Simone Haupt (Life and Brain GMBH), Carlos Villaescusas (Novo Nordisk).

● Invited talks:	na
● Science communication:	NICO UNIGHT INTOtheBRAIN connessioni che non ti aspetti tra inquinamento, vivere green e cervello (UNIGHT il 30 settembre torna la Notte europea dei ricercatori (unito.it)) NICO Virtual Tour (interview on Lightsheet microscopy)
● Editorial duties:	Ad hoc reviewer for Neurochemical Research, Journal of the Neurological Sciences and Frontiers Aging Neuroscience.
● others	Poster participation: Boda E, Lorenzati M, **Parolisi R**, Harding B, Pallavicini G, Bonfanti L, Moccia A, Bielas S, Di Cunto F, Buffo A (2022) *Molecular and functional heterogeneity in dorsal and ventral oligodendrocyte progenitor cells of the mouse forebrain in response to DNA damage. FENS Meeting, Paris, July 2022* Bonato M, Montarolo F, **Parolisi R**, Pandino C, Bertolotto A, Buffo A, Boda E. *Selective behavioral alterations after acute particulate matter exposure in a pre-symptomatic Multiple Sclerosis mouse model.* NATIONAL MEETING OF PHD STUDENTS IN NEUROSCIENCE 2022, SINS event (11 June, Brescia) Bonato M, Montarolo F**, Parolisi R**, Pandino C, Bertolotto A, Buffo A, Boda E. *Selective behavioral alterations after acute particulate matter exposure in a pre-symptomatic Multiple Sclerosis mouse model.* 5th Brainstorming Research Assembly for Young Neuroscientists (BraYn congress) 2022 (28-30 September, Rome). Gómez-González GB, Bessuso D, Zucca S, **Parolisi R**, Ribodino M, Dheghan H, Scaramuzza L, Cattaneo E, Buffo A. *Assessing the functional integration of hESC-derived striatal grafts in a rat model of Huntington Disease by calcium photometry: pilot experiments on S1FL cortex.* NSC-Reconstruct Annual Meeting Program, Bellagio (Italy) Participation in the NSC-Reconstruct Annual Meeting Program in Bellagio (Italy), April 5-7th 2022.
Organizational activities and responsibilities at NICO:	In charge of the maintenance of EM and 3D lightsheet microscopy; and one of the confocal microscopes, hosted at NICO microscopy facility.
Speakers invited:	Ludovico Silvestri (LENS - European Laboratory for Non-Linear Spectroscopy, University of Florence).
Other organizational activities:	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Valentina Cerrato, senior Post Doc[31]

Supervised PhD students:	-

[31] Please duplicate the module for the various lab members

Honors, prizes, awards:	-
Outreach activities	
● International collaborations:	Prof. Ludovic Telley (University of Lausanne, Switzerland); Prof. Magdalena Götz (University of Munich, Germany)
● Invited talks:	- "*The multiple levels of astrocytes' heterogeneity: a lesson from the cerebellum*" 24 Sept 2022, SINAPSI Forum Neuroscienze, University of Turin, Turin. - "*The multiple levels of cerebellar astrocytes heterogeneity: from developmental trajectories, to spatial patterning and functional interactions with specific neuronal circuitries*", 15 Dec 2022, III More Than Neurons Meeting, Turin, Italy
● Science communication:	NICO Virtual Tour (interview on confocal microscopy)
● Editorial duties:	Ad hoc reviewer for Cells, Brain Sciences, Frontiers in Cellular Neuroscience, Glia, International Journal of Developmental Neuroscience, International Journal of Molecular Sciences, Neurochemical Research, Neural Regeneration Research, Neuroscience
● others	Poster participation: Lorenzati M, Nicorvo E, Ribodino M, Signorino E, **Cerrato V**, Grimaldi P, Berchialla P, Conti L, Cortelli P, Giorgio E, Buffo A. *Human iPSCs-derived oligodendrocytes and astrocytes as the first Autosomal Dominant Leukodystrophy-relevant cellular model.* FENS Meeting, July 2022 Membership of Scientific Societies: Italian Society of Neuroscience (SINS)
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. Carmen Falcone (SISSA, Trieste)
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Giulia Nato, senior PostDoc

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:	na
● Science communication:	na
● Editorial duties:	na
● others	

Organizational activities and responsibilities at NICO:	Co-responsible of Leica SP5 confocal microscopy training
Speakers invited:	na
Other organizational activities:	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Martina Lorenzati, PostDoc

Supervised PhD students:	n.a.
Honors, prizes, awards:	-
Outreach activities	
● International collaborations:	-
● Invited talks:	-
● Science communication:	"Vivere per sempre", Giovedì Scienza (March 2022) – announcer&speaker "Pint of Science" – co-responsible of "Beautiful Mind" Theme (May 2022) "NICO Virtual Tour" (interview on human iPSC cell cultures)
● Editorial duties:	-
● others	<u>Posters at meetings:</u> **Lorenzati M**, Nicorvo E, Ribodino M, Signorino E, Cerrato V, Grimaldi P, Berchialla P, Conti L, Cortelli P, Giorgio E, Buffo A. *Human iPSCs-derived oligodendrocytes and astrocytes as the first Autosomal Dominant Leukodystrophy-relevant cellular model.* FENS Meeting, July 2022 Boda E, **Lorenzati M**, Parolisi R, Harding B, Pallavicini G, Bonfanti L, Moccia A, Bielas S, Di Cunto F, Buffo A (2022) *Molecular and functional heterogeneity in dorsal and ventral oligodendrocyte progenitor cells of the mouse forebrain in response to DNA damage.* FENS Meeting, Paris, July 2022 Ribodino M, **Lorenzati M**, Signorino E, Nicorvo E, Grimaldi P, Conti L, Cortelli P, Berchialla P, Giorgio E, Buffo A. *Human IPSCS-derived oligodendrocytes and astrocytes as the first autosomal dominant leukodystrophy-relevant cellular models.* National Meeting of PhD students in Neuroscience, 11 June 2022, Brescia, Italy <u>Membership of Scientific Societies:</u> Italian Society of Neuroscience (SINS)
Organizational activities and responsibilities at NICO:	Co-Responsible for the Dissection Room at NICO
Speakers invited:	-
Other organizational activities:	-

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

Gabriela B Gomez Gonzalez, PostDoc

Supervised PhD students:	-
Honors, prizes, awards:	-
Outreach activities	
● International collaborations:	NSC-Reconstruct Partners: Elena Cattaneo, Coordinator (Università di Milano), Malin Parmar (University of Lund), Oliver Bruestle (UniversitaetsKlinikum Bonn), Ernest Arenas (Karolinska Institutet), Roger Barker (University of Cambridge), Agnete Kirkeby (Kobenhavns Universitet), Meng Li (Cardiff University), Magdalena Goetz (Helmholtz Zentrum Muenchen), Pierre Vanderhaeghen (VIB-KULeuven Center) , Andreas Bosio (Miltenyi Biotec GMBH), Simone Haupt (Life and Brain GMBH), Carlos Villaescusas (Novo Nordisk). Prof. Ataúlfo Martínez Torres (Universidad Autonoma de Mexico, Institute of Neuroscience)
● Invited talks:	
● Science communication:	
● Editorial duties:	-
● others	NSC-Reconstruct **Annual Meeting, Bellagio,** Italy. April 5-7th 2022. **Poster:**Assessing the functional integration of hESC-derived striatal grafts in a rat model of Huntington Disease by calcium photometry: pilot experiments on S1FL cortex. Gómez-González GB, Bessuso D, Zucca S, Parolisi R, Ribodino M, Dheghan H, Scaramuzza L, Cattaneo E, Buffo A. **Remote Fiber Photometry Workshop.** Standford University. USA. August 25th, Sept 1st 2022. Organized by Dr. Deisseroth K Lab. **Virtual Miniscope Workshop**. MetacCell- UCLA, USA. Nov 29-Dec 2nd 2022. Organized by Daniel Aharoni, Denise Cai, and Tristan Shuman.
Organizational activities and responsibilities at NICO:	Organizer of Buffo's Lab internal seminars
Speakers invited:	Dr. Ben Vermaercke: Laboratory of Stem Cell and Developmental Neurobiology (VIB-KU Leuven) Feb 24th, 2022. Title: "the functional integration of human transplanted neurons in the mouse visual cortex"
Other organizational activities:	-
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	-

Maryam Khastkhodaei , PhD student

Supervised PhD students:	na
Honors, prizes, awards:	-
Outreach activities	
● International collaborations:	-
● Invited talks:	BraYn 2022-Fifth brainstorming research assembly for young neuroscientists, September 28th-30th 2022, Rome, Italy. Oral presentation: *Rescuing neural cell survival and maturation in a primary autosomal recessive microcephaly-17 (MCPH17) mouse model: effects of the postnatal N-acetyl cysteine treatment.* SINS PhD meeting- Italian Society for Neuroscience - June 11th, 2022, Brescia, Italy. Oral presentation: *Rescuing neural cell survival and maturation in a primary autosomal recessive microcephaly-17 (MCPH17) mouse model: effects of the postnatal N-acetyl cysteine treatment.*
● Science communication:	-
● Editorial duties:	-
● others	**Courses:** How we reason - *M.Bucciarelli , May 18th and 25th 2022.* Observational and Experimental Studies for Therapeutic Interventions in Movement Disorders - *Aristide Merola, February 17th, March 10th and 18th 2022.* Open Science A to Z - *Elena Giglia- PhD course, 17-19 January 2022 (12 hours), ISPAS project, European Union's Horizon 2020 research and innovation program.* FAIR DATA BASICS - *Elena Giglia- PhD course, 16-18 February 2022 (9 hours), ISPAS project, European Union's Horizon 2020 research and innovation program.* Italian language course, A1-A2 - *Elena Laura Baratono- PhD course for international students, March 2nd- May 16th 2022.* **Workshops:** Research professional: Fund your research - *Isidoro Riondato, Research Promotion Unit, June 9th, 2022.* Single cell sequencing technologies & applications, *Human technopole training, September 13th 2022. (Online event)* Astrocyte Café - *Series of monthly meetings about astrocyte research* Motor Neuron Diseases*: understanding the pathogenetic mechanisms to develop therapies, November 4th-5th 2022.* Public engagement and outreach , *November 18th-19th 2022.* **Conferences:** SINS PhD meeting- Italian Society for Neuroscience **-** June 11th, 2022, Brescia, Italy FENS Forum 2022, July 9th-13th, 2022, Paris, France BraYn 2022-Fifth brainstorming research assembly for young neuroscientists, September 28th-30th 2022, Rome, Italy **Membership of Scientific Societies:**

	Federation of the European Neuroscience Societies (FENS) Italian Society of Neuroscience (SINS) Brainstorming Research Assembly for Young Neuroscientists (BraYn) Association
Organizational activities and responsibilities at NICO:	-
Speakers invited:	-
Other organizational activities:	-
Workshops, Schools or Conferences organized:	-
Technology transfer achievements (patents, etc.):	-

Martino Bonato, PhD Student

Supervised PhD students:	
Honors, prizes, awards:	
Outreach activities	
● International collaborations:	
● Invited talks:[32]	
● Science communication:[33]	- UNITO UNIGHT INTOtheBRAIN connessioni che non ti aspetti tra inquinamento, vivere green e cervello, Caffè Scientifico alla Casa del Quartiere ((Notte dei ricercatori, vivere green e gli effetti benefici sul cervello- Corriere.it)
● Editorial duties:	
● others	Posters at meetings: **Bonato M**, Montarolo F, Parolisi R, Pandino C, Bertolotto A, Buffo A, Boda E. *Selective behavioral alterations after acute particulate matter exposure in a pre-symptomatic Multiple Sclerosis mouse model.* SINS PhD meeting- Italian Society for Neuroscience - June 11th, 2022, Brescia, Italy **Bonato M**, Montarolo F, Parolisi R, Pandino C, Bertolotto A, Buffo A, Boda E. *Selective behavioral alterations after acute particulate matter exposure in a pre-symptomatic Multiple Sclerosis mouse model.* 5th Brainstorming Research Assembly for Young Neuroscientists (BraYn congress) 2022 (28-30 September, Rome)
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Other organizational activities:[34]	
Workshops, Schools or Conferences organized:	

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

[33] Public engagement

[34] No university appointments.

Technology transfer achievements (patents, etc.):	

Marta Ribodino, PhD student

Supervised PhD students:	
Honors, prizes, awards:	
Outreach activities	
● International collaborations:	NSC-Reconstruct Partners including Malin Parmar group, University of Lund.
● Invited talks:	
● Science communication:	
● Editorial duties:	
● others	Poster presentation or participation: **Ribodino M**, Lorenzati M, Signorino E, Nicorvo E, Grimaldi P, Conti L, Cortelli P, Berchialla P, Giorgio E, Buffo A. Human IPSCS-derived oligodendrocytes and astrocytes as the first autosomal dominant leukodystrophy-relevant cellular models. National Meeting of PhD students in Neuroscience, 11 June 2022, Brescia, Italy Lorenzati M, Nicorvo E, **Ribodino M**, Signorino E, Cerrato V, Grimaldi P, Berchialla P, Conti L, Cortelli P, Giorgio E, Buffo A. *Human iPSCs-derived oligodendrocytes and astrocytes as the first Autosomal Dominant Leukodystrophy-relevant cellular model.* FENS Meeting, July 2022 Attended meetings: NSC-Reconstruct meeting, 5-7 April 2022, Bellagio, Italy NECTAR conference, 24-26 October 2022, Athens, Greece
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Ersilia Nicorvo, research fellow

Supervised PhD students:	
Honors, prizes, awards:	
Outreach activities	
● International collaborations:	
● Invited talks:	
● Science communication:	
● Editorial duties:	
● others	Poster participation: - Ribodino M, Lorenzati M, Signorino E, **Nicorvo E**, Grimaldi P, Conti L, Cortelli P, Bechialla P, Giorgio E,

	Buffo A. Human IPSCS-derived oligodendrocytes and astrocytes as the first autosomal dominant leukodystrophy-relevant cellular models. National Meeting of PhD students in Neuroscience, 11 June 2022, Brescia, Italy - Lorenzati M, **Nicorvo E**, Ribodino M, Signorino E, Cerrato V, Grimaldi P, Berchialla P, Conti L, Cortelli P, Giorgio E, Buffo A. *Human iPSCs-derived oligodendrocytes and astrocytes as the first Autosomal Dominant Leukodystrophy-relevant cellular model.* FENS Meeting, July 2022
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

4. Research activity in 2022[35]

a. Summary (500 characters)

In 2022, our group demonstrated that the developmental heterogeneity influences the response of forebrain oligodendrocyte progenitor cells (OPCs) to accumulation of DNA damage due to ablation of Citron-kinase (Cit-k) or cisplatin treatment (Boda et al., 2022). In a collaborative study, we also investigated curcumin-based fluorescent probes able to bind to aldehyde dehydrogenase 1A3 (ALDH1A3) as tools for the identification of the glioblastoma mass in vivo (Gelardi et al., 2022).

b. Background and rationale (3000 characters)

In 2022 we have put our main focus on the investigation of glia physiopathology and of issues linked to the establishment of successful regenerative medicine approaches.

In several demyelinating pathologies, such as Multiple Sclerosis (MS), OPCs are not able to support efficient myelin regeneration. Dysregulation of cell intrinsic and extrinsic mechanisms regulating OPC/oligodendrocyte biology (i.e. survival, morphology, proliferation and differentiation) underlies such inability. The identification of such mechanisms/factors is a hot topic in the field, as it may provide targets to design preventive/therapeutic interventions. In this context, an additional element of complexity resides in oligodendroglia heterogeneity, whose impact on OPC/oligodendrocyte (OL) response to injuries remains to be investigated. Moreover, OLs are primarily hit in a number of genetic hypo-/dys-myelinating disorders, i.e. leukodystrophies. Among these pathologies, Autosomal Dominant Leukodystrophy (ADLD) is a rare disease leading to CNS white matter loss, caused by the duplication of the LMNB1 gene, which codes for a structural protein located in the nuclear lamina. Based on the evidence showing glial pathology in ADLD patients, a human glial cell model from ADLD patients is required to study the mechanistic aspects of this pathology.

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

Astrocytes (AS) comprise extremely heterogeneous types. In the past we have unveiled fundamental cellular mechanisms implicated in the generation of the diversity of cerebellar AS (Cerrato et al., PLoS Biol 2018) and identified two non-overlapping postnatal progenitor pools which generate different types of cerebellar AS (Kantzer et al., J Neurosci Res, 2021). However, much remains to be understood on the molecular actors of AS specification and on the molecular and functional heterogeneity of mature AS. Prospective markers to distinguish distinct differentiation potencies or stages in AS lineages are missing and may facilitate more in-depth studies such as single cell profiling on both mouse and human cerebella aimed at disentangling the emergence of such diversity. While the development and functions of the cerebellum have been well studied in animal models, our understanding of the human cerebellum remains limited. Moreover, despite extensive study of cerebellar neurons, the development, maturation, and functions of cerebellar glial cells remain largely unknown, especially in humans. The study of the human cerebellum and of the involvement of glial cells in its developmental dynamics represent very important aspects and probe the basis to better understand its functioning and disease.

In the effort to develop regenerative approaches for Huntington's disease (HD), we reasoned that exposure to environmental enrichment well known to stimulate neuroplasticity could increase the therapeutic efficacy of human striatal grafts implemented in a rat HD model. In parallel studies we aimed to define the cell type identity of transient new neurons produced in mouse by striatal AS reacting to excitotoxic injury.

c. Objectives (1000 characters)

We have investigated the neurobiology of glial cells and worked to devise strategies for cell replacement with the ultimate goal to identify cellular and molecular targets to promote repair in acute and chronic neurodegenerative pathologies. During 2022 we specifically aimed to: (a) identify intrinsic factors affecting OPC response to damage; (b) define strategies to rescue diseased OL phenotypes; (c) disclose how astrocytes heterogeneity is achieved.

d. Results (4000 characters)

In 2022 we completed the revisions of one study (Boda et al., 2022) and submitted two new manuscripts. We also contributed to one other collaborative paper (Gelardi et al., 2022), as connected to our interests and know-how.

In Boda et al. 2022 (Nat Communications, doi: 10.1038/s41467-022-30010-6), we found that developmental heterogeneity influences the response of forebrain OPCs to accumulation of DNA damage due to ablation of Citron-kinase (Cit-k) or cisplatin treatment. Specifically, by exploiting germinal and conditional Cit-k mutants, as well as in vitro assays, we showed that OPCs derived from dorsal Emx1+ precursors are intrinsically more vulnerable to DNA damage and undergo cell death, while OPCs originated from ventral Nkx2.1 precursors are more resilient and persist in the tissue, although entering a senescent-like state. Such alternative fates are associated with a different activation of NRF2-mediated anti-oxidant responses.

In Grimaldi*, Lorenzati* et al. (Grimaldi P*, Lorenzati M*, Ribodino M, Signorino E, Buffo A**, Berchialla P**, "Predicting astrocytic nuclear morphology with machine learning: A tree ensemble classifier study", submitted to Applied Biosciences; * co-first, ** co-last), we report about the establishment of a protocol to generate human glial cells (astrocytes and oligodendrocytes) from human-induced pluripotent stem cells (hiPSCs) and about the development of a supervised Machine Learning approach to classify astrocytes based on nuclear morphologies. We found that LMNB1 intensity, nuclear area, cellular area and the number of ramifications as important predictors in the classification process. This model provides a novel tool to investigate the effects of LMNB1 expression dysregulation, responsible of ADLD, in glial cells.

In 'Schellino R, Besusso D, Parolisi R, Gómez-González G, Dallere S, Scaramuzza L, Ribodino M, Campus I, Conforti P, Parmar M, Boido M, Cattaneo E, Buffo A. Enriched environment promotes long-term human

striatal graft maturation, circuit reconstruction and motor recovery in a rat model of Huntington's disease, SCRT-D-23-00062, submitted to Stem Cell Research and Therapy', we report that grafted human striatal cells survived up to 6 months after transplantation and showed morphological and neurochemical features typical of human medium spiny neurons (MSNs). Donor derived interneurons were also detected. Grafts wired in both local and long-range striatal circuits and formed domains suggestive of distinct striosome-like territories. Of note, complex motor performances affected by QA were significantly improved by the grafts. Moreover, exposure to enriched environment (EE) selectively increased MSN differentiation, promoted host- to-graft connectivity and further enhanced task execution. These data demonstrate the long-term therapeutic potential of ESC-derived grafts for HD and suggest that EE can effectively accelerate the maturation and promote the integration of human striatal neurons.

In a collaborative study (Gelardi et al., 2022, S. Biol, doi: 10.1038/s42003-022-03834-7), we contributed to validate curcumin-based fluorescent probes able to bind to aldehyde dehydrogenase 1A3 (ALDH1A3) as tools for the identification of glioblastoma mass in vivo.

e. Advancement in the field (1000 characters)

Among published papers, we foresee that the following findings will have the major impact: (i) Evidence that developmental heterogeneity influences OPC response to injury and that this is explained by divergent anti-oxidant responses regulated at the post-transcriptional level; (ii) Employment of bona fide predictive markers to classify astroglial morphologies in vitro and investigate astroglial pathology as related to LMNB1 dysregulation of LMNB1 protein levels such as ADLD and senescence.

f. Publications[36]

Boda E, Lorenzati M, Parolisi R, Harding B, Pallavicini G, Bonfanti L, Moccia A, Bielas S, Di Cunto F, **Buffo A** (2022) Molecular and functional heterogeneity in dorsal and ventral oligodendrocyte progenitor cells of the mouse forebrain in response to DNA damage. Nat Comm 28 April 2022; doi.org/10.1038/s41467-022-30010-6

Magrassi L, **Nato G**, Delia D, **Buffo A**. (2022) Cell-Autonomous Processes That Impair Xenograft Survival into the Cerebellum. Cerebellum. 21:821-825. doi: 10.1007/s12311-022-01414-3

Gelardi ELM, Caprioglio D, Colombo G, Del Grosso E, Mazzoletti D, Mattoteia D, Salamone S, Ferraris DM, Aronica E, **Nato G, Buffo A**, Rizzi M, Magrassi L, Minassi A, Garavaglia (2022) Curcumin-based-fluorescent probes targeting ALDH1A3 as a promising tool for glioblastoma precision surgery and early diagnosis. S. Biol. 5:895. doi: 10.1038/s42003-022-03834-7.

Boda E, Boscia F, Lohr C (2022) Editorial: The Role of Astroglia and Oligodendroglia in CNS Development, Plasticity, and Disease – Novel Tools and Investigative Approaches Front. Cell. Neurosci., 09 May 2022 | doi.org/10.3389/fncel.2022.901820

[36] DO NOT include papers in press or submitted.

5. 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 OL studies, we will specifically focus on pharmacological approaches targeting oxidative stress, as a possible therapeutic strategy in a mouse model of microcephaly 17 (MCPH17), a neurodevelopmental disorder associated with Cit-K mutations. In addition, having established human OL and AS cellular models derived from pluripotent stem cells, we will investigate the basis of leukodystrophies in patient-derived cells and validate the therapeutic value of an allele silencing strategy which we have proved effective in other models.

The investigation of AS heterogeneity will proceed through multi-omic analyses of adult mouse cerebellar cells, by integrating publicly available and our original single nuclei/single cell RNA sequencing datasets. Focus will be on the spatial topography of the cerebellum in view of highlighting features of each AS type specific to its interplay with defined neuronal circuits/subtypes. This will be also achieved through high throughput innovative spatial transcriptomics experiments. Moreover, we will focus specifically on cerebellar nuclei astrocytes, whose molecular features and physiological properties are still completely unknown.

In regenerative medicine studies, we will start working to record and modulate graft activity in vivo. In parallel, through comparison of the transcriptional profiles of different striatal lineages, we will delineate the identity of new neurons produced in mouse by AS reacting to excitotoxic injuries.

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

Fundamental issues on glia biology are poorly understood: (i) mechanisms regulating OPC maturation, myelin repair and disease; (ii) distinction, specification and maintenance 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 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:

- We discovered that Cit-k loss leads to specific dysfunctions in OL subsets leading to apoptosis or cell senescence, and cell-autonomously determining hypomyelination (Boda et al., 2022). The long-term functional consequences remain to be understood. In addition, in the Cit-k KO model of primary microcephaly 17 (MCPH17) we found prominent alterations of all glial types which are associated with strong oxidative stress. No treatment or cure is currently available for MCPH17, other primary or secondary microcephaly, and associated neurodevelopmental defects. We propose to study if early developmental antioxidant treatments can modify the disease trajectory.

- ADLD is characterized by myelin loss and OLs are considered as the main target of LMNB1 duplication. Yet, other data point also to astrocyte dysfunction. On these bases in 2022 we generated astrocytes and oligodendrocytes from ADLD patient- and healthy donor (CTRL)- derived human-induced pluripotent stem cells (hiPSCs). Investigations of astrocytes -which we produced more efficiently compared to

oligodendrocytes-, revealed that ADLD AS display an altered expression of LMNB1 both at RNA and protein level, together with nuclear shape alterations associated to defined morphological and neurochemical cell features (e.g. cell area, nuclear area and GFAP protein levels). Moreover, bulk RNAseq analysis on ADLD AS revealed pathological alterations in extracellular matrix production, calcium ion homeostasis in response to glutamate signaling and RNA processing. If and how these ADLD AS dysfunctions take part in ADLD OL pathology and if they can be rescued is currently under investigation.

- We focused on the investigation of the development of AS heterogeneity in the mouse cerebellum through single nuclei/single cell (sn/sc) RNA sequencing and distinguished the transcriptome of the main astrocyte types, thus uncovering both cell intrinsic and extrinsic mechanisms of cell fate determination. Moreover, we uncovered the molecular signature of cerebellar nuclei AS, so far never investigated, which showed a separate developmental trajectory and a unique neurochemical profile compared to the other subtypes. However, much remains to be understood on the molecular actors of AS types specification and on the role of cerebellar nuclei AS in cerebellar development and functioning.

- We showed that grafted human striatal cells survived up to 6 months after transplantation and about 10% acquired features typical of hMSNs. Donor derived interneurons were also detected. Grafts wired in both local and long-range striatal circuits and formed domains suggestive of distinct striosome-like territories. Of note, complex motor performances affected by QA were significantly improved by the grafts. Moreover, exposure to enriched environment (EE) selectively increased MSN differentiation, promoted host- to-graft connectivity and further enhanced task execution (Schellino, submitted). Also, our collaborators set up a protocol leading to hMSN with increased yield and maturity (second generation human MSN, sg-hMSN) which is now ready to be tested in vivo.

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

In 2023 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 and can be exploited to treat disease;

- optimizing cell replacement approaches focused on the re-installment 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 identifying mechanisms to be fostered or manipulated in view of proposing preclinical therapeutic approaches for diseases such as Leukodystrophies, MS and microcephaly; (ii) use innovative experimental models derived from hiPSCs to better understand disease and and pave the way for future CNS cell replacement therapies for neurodegenerative pathologies.

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

We will pursue the following aims:

Cellular and molecular mechanisms of oligodendroglial dysfunctions

- As a follow up of findings showing partial tissue recovery in the Cit-k KO mouse model of MCPH17 upon NAC (an FDA approved glutathione precursor) treatment, in 2023 we will investigate if and to what extent NAC administration starting during either embryonic and postnatal development can revert neuroanatomical, functional, behavioral traits and the short life span of the mouse mutants. Based on already collected data showing efficient integration, expansion and initial maturation of WT OPCs in the diseased Cit-K KO brain,

we will also test the hypothesis that replacement of this glial population, which is dramatically impaired by the mutation, alone or in combination with NAC treatment, can exert an efficient rescue of the pathology and extend mutant mice survival (Khastkhodaei, in preparation).

In parallel, we will be exploring the long-term effect in vivo of Cit-k KO deletion and DNA damage in OL-specific Cit-k KO mice and we will set the stage to address the cellular and molecular mechanisms differentially imprinting dorsal and ventral mouse OPC antioxidant responses in the presence of DNA damage. Coll: F Di Cunto, M. Boido and S. Bovetti (NICO) and Prof. F Ferrini, University of Turin.

- After characterization of the pathological profile of ADLD AS, in 2023 we will work on the validation of the efficacy of silencing of excessive LMNB1 production to revert the cellular pathology. We will use an Allele SPecific Silencing (ASP-siRNA) strategy, already established in ADLD fibroblasts, iNeurons and OPCs overexpressing human LMNB1 in Giorgio*, Lorenzati et al (2019). Moreover, we will set up a number of co-culture assays to prove the detrimental effects of dysfunctional ADLD AS on oligodendroglia and confirm the reversion of these actions by ASP-siRNA (Lorenzati et al., in preparation). Coll: Proff. E Giorgio, Univ Pavia; Prof. P Cortelli, Univ of Bologna.

Identification of determinants of astrocyte diversity

- We will pursue the overarching goal of reconstructing the ontogenesis of AS heterogeneity during mouse cerebellar development. To this aim, genetic fate mapping will be used to address whether cerebellar nuclei AS share the same origin with the other cerebellar astrocytes subtypes or are born from a separate progenitor pool, while single nuclei/single cell RNA sequencing and spatial transcriptomics will reveal the molecular dynamics of the origin and differentiation of cerebellar AS diversity with an unprecedented temporal and spatial resolution (Cerrato et al., in preparation). Pilot snRNA sequencing experiments will be also performed to address the same issue in human fetal samples. Coll: Prof L Telley, Univ Lausanne.

- We will provide the first molecular and functional characterization of AS of the cerebellar nuclei. This will be achieved through *ex vivo* and *in vivo* targeted cell manipulation experiments through both pharmacological and viral approaches coupled with electrophysiological recordings and behavioral experiments. Coll: Prof L Telley, Univ Lausanne; Prof. M. Götz, LMU; Prof. M.C. Miniaci, Univ Naples.

Development of cell replacement approaches for Huntington Disease
In this research line focus will be on (i) how the graft becomes integrated in the host circuits and improves behavior by recording graft calcium transients in vivo and by manipulating graft activity by optogenetic and chemogenetic approaches. This task will be first addressed in rat allografts and then transferred to human xenografts; (ii) assessing the integration and maturation potential in vivo of sg-hMSNs shown to have a high hMSN yield in vitro (Conforti et al., 2022). Finally, to understand mouse striatal AS neurogenic potential, we will conclude the phenotyping of neuroblasts generated in mouse striatum after excitotoxic lesion and report on the dynamic features of the neurogenic clones established by AS in this system (Nato et al., in preparation; Fogli et al., in preparation). Coll: Proff F Luzzati, NICO; E Cattaneo, Univ of Milano.

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

Several of the addressed questions (eg identifying molecular substrates of OL biology in health and disease, understanding the emergence of AS heterogeneity and the nature of AS neurogenic competence) are fundamental research questions essentially unanswered.

A further fundamental unanswered research question is about the extent and quality of the plastic properties of the diseased mature brain: can circuits such as those of the striatum be faithfully reconstructed? Can this be achieved based on the integration of human neurons produced in vitro? If so, to what extent can striatal functions be rescued and via what mechanisms? On these bases, our studies will provide unique insight to the related evolving fields.

Our approaches (eg. bioinformatic approaches, gene expression analyses on select cell populations and on human cells, development of new mutant lines, human cell models and in vivo neuronal activity recording/manipulation) represent cutting edge techniques whose integration confers methodological originality to our studies. We are among the few labs at our University to have implemented these technologies and to combine them in our research work.

Also, focus on the cerebellum provides a specific advantage in the field of AS diversity (well established for this territory), reprogramming and prospectively generation of hPSC-derivatives.

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. A line of potential innovation will come by the need to develop and/or import dedicated algorithms for spatial transcriptomics analysis (EASI Genomics Grant).

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2022

Laboratory name: **Embryonic neurogenesis**

1. LABORATORY DESCRIPTION – PERSONNEL:[37]

Principal Investigator
Di Cunto, Ferdinando, Full Professor, MD-PhD, 20/12/1969, 011 6706616, ferdinando.dicunto@unito.it

Personnel
Pallavicini, Gianmarco, Post-doctoral Fellow, PhD in Molecular Biotechnology, 10/10/1991, 011 6706616, gianmarco.pallavicin@unito.it, Molecular biology and biochemical analysis of genetically modified cellular and mouse models of microcephaly and brain tumors.

Pritz, Christian, Post-doctoral Fellow, PhD in Molecular Biology, 30/08/1983, 011 6706616, christian.pritz@unito.it, Generation and analysis of C. elegans models of brain diseases.

Iegiani, Giorgia, PhD Student, MSc in Molecular Biotechnology, 17/04/1996, , 011 6706616, giorgia.iegiani@unito.it, Molecular biology and biochemical analysis of genetically modified cellular and mouse models of microcephaly and brain tumors.

Ferraro, Alessia, PhD Student, MSc in Biotechnology, 22/08/1997, 011 6706616, alessia.ferraro@unito.it, of genetically modified cellular and mouse models of microcephaly and brain tumors.

Onorato, Giada, PhD Student, MSc in Biology, 29/01/1993, 011 6706616, giadaonorato93@gmail.com, Generation and analysis of C. elegans models of brain diseases.

2 CURRENT GRANTS

Starting-end date	Project Title and ID	Beneficiary[38]	Funding Program/Agency	Role of the unit[39]	Overall Amount Funded	Managed by FCO/UNITO
01/01/2020 30/06/2025	Development of Citron Kinase as a therapeutic target for brain tumors. IG 23341	PI	AIRC	Coordinator	855000	01/01/2020 30/06/2025
01/04/2022 31/03/2024.	Study of primary microcephaly genes as therapeutic targets for	Gianmarco Pallavicini	AIRC fellowship	Coordinator	50000	01/04/2022 31/03/2024.

[37] For further personnel copy the corresponding form, and number accordingly; do not exceed one line to describe role & expertise
[38] 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.
[39] Coordinator/PI of research unit/team component.

	glioblastoma multiforme"					

3 SCIENTIFIC ACTIVITIES IN 2022

Name, Role (PI)

Supervised PhD students:	Giorgia Iegiani, Giada Onorato, Alessia Ferraro
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. - Dr. Yohann Couté, Laboratoire Biologie à Grande Echelle, Biosciences and Biotechnology Institute of Grenoble, France - Prof. Shaun Stauffer, Center for Therapeutics Discovery, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio 44195, United States.
• Invited talks:[40]	
• Science communication:[41]	17/03/2020 – Present: Personal Scientific blog for general public on COVID-19 pandemics: https://standandfightthevirus.blogspot.com/
• Editorial duties:	- Associated Editor of PLoS ONE - Associated Editor of Frontiers in Neurogenesis
• others[42]	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); Bioinformatics Italian Society (BITS)
Organizational activities and responsibilities at NICO:	Data Management
Speakers invited:	
Other organizational activities:[43]	
Workshops, Schools or Conferences organized:	President of the Organizing Committee of the XX meeting of SINS (Società Italiana di Neuroscienze)
Technology transfer achievements (patents, etc.):	

Gianmarco Pallavicini (Post-doctoral fellow)

Supervised PhD students:	Giorgia Iegiani, Alessia Ferraro
Honors, prizes, awards:	BRAYN 2022 – session chairmen
Outreach activities	

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

• International collaborations:	- Visiting scientist in the laboratory of Prof. Stephanie Bielas, Department of Human Genetics, University of Michigan Medical School, Ann Arbor, Michigan, USA.
• Invited talks:	-SINAPSI forum, 24th September 2022 Torino Italy - BRAYN 2022, 5th Brainstorming Research Assembly for Young Neuroscientists - 28th to 30th September 2022, Rome, Italy. - 6th Quadrennial Meeting of the World Federation of Neuro-Oncology Societies (WFNOS 2022) - March 24 to 27 2022, Seul, Corea (online meeting)
• Science communication:	-Poster presentation - Brain Tumor Meeting 2022 May 19-20, Berlin, Germany

Giorgia Iegiani (PhD student)

Outreach activities	
• International collaborations:	- Visiting student in the laboratory of Prof. Stephanie Bielas, Department of Human Genetics, University of Michigan Medical School, Ann Arbor, Michigan, USA.
• others	- WFNOS 2022 - 6th Quadrennial Meeting of the World Federation of Neuro-Oncology Societies () - March 24 to 27 2022, Seul, Corea (online participation) - Brain Tumor Meeting 2022 , May 19-20, Berlin, Germany - ENCODS 2022, European Neuroscience Conference by Doctoral Students - July 7th & 8th, 2022 at Collège de France, Paris, France - BRAYN 2022 5th Brainstorming Research Assembly for Young Neuroscientists - 28th to 30th September 2022, Rome, Italy - ASGH 2022 American Society of Human Genetics Annual Meeting in Los Angeles, California, October 25-29, 2022
Other organizational activities:	President of the National Italian Biotechnologists Association (ANBI)

Giada Onorato (PhD Student)

Workshops, Schools or Conferences organized:	Participation to The ESA-FAIR Space Radiation School 2022- Darmstadt (Germany), 4-20 September 2022

4. Research activity in 2022[44]

g. 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.

h. 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. In the last few years, we have underscored that most of the biological activities of CITK can be reconciled with the modifications which it produces on the dynamics of microtubule cytoskeleton. For these reasons, we are investigating how microtubule-dependent events may simultaneously affect mitotic fidelity and genomic integrity. On this line, our latest achievement was to demonstrate a functional interplay between CITK and Kinesin Binding Protein 1 (KIF1BP), a microtubule related protein previously implicated in a complex microcephaly syndrome. 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.

i. 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. what is the mechanism linking CITK, KIF1BP and microtubule remodeling;
4. CITK as a possible target for cancer therapy.

j. 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

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

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. Goldberg-Shprintzen disease (GOSHS) is a rare microcephaly syndrome accompanied by intellectual disability, dysmorphic facial features, peripheral neuropathy and Hirschsprung disease. It is associated with recessive mutations in the gene encoding kinesin family member 1-binding protein (KIF1BP, also known as KIFBP). The encoded protein regulates axon microtubules dynamics, kinesin attachment and mitochondrial biogenesis, but it is not clear how its loss could lead to microcephaly. We identified KIF1BP in the interactome of CITK. KIF1BP and CITK interact under physiological conditions in mitotic cells. Similar to CITK, KIF1BP is enriched at the midbody ring and is required for cytokinesis. The association between KIF1BP and CITK can be influenced by CITK activity, and the two proteins may antagonize each other for their midbody localization. KIF1BP knockdown decreases microtubule stability, increases KIF23 midbody levels and impairs midbody localization of KIF14, as well as of chromosome passenger complex. These data indicate that KIF1BP is a CITK interactor involved in midbody maturation and abscission, and may play a crucial role downstream of CITK also in microtubule remodeling and prevention of DNA double strand breaks accumulation.
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.

k. Advancement in the field (1000 characters)

The results which we obtained have contributed important advances in the field of microcephaly studies. In addition, we have provided evidence that genes involved in primary microcephaly are suitable targets for brain tmor therapy, in particular medulloblastoma.

l. Publications[45]

1. Boda E, Lorenzati M, Parolisi R, Harding B, Pallavicini G, Bonfanti L, Moccia A, Bielas S, Di Cunto F, Buffo A. 2022 Molecular and functional heterogeneity in dorsal and ventral oligodendrocyte progenitor cells of the mouse forebrain in response to DNA damage. Nat Commun. 2022 Apr 28;13(1):2331. doi: 10.1038/s41467-022-30010-6

5. Future directions and objectives for next years

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

a. Summary (up to 2000 characters):

During the next three years, we plan to continue the development of the current research lines and in particular:
1. We will continue to dissect the molecular mechanisms by which Citron kinase loss leads to microcephaly. To this regard, we plan to study the mechanisms by which CITK loss alters microtubule nucleation and stability. Moreover, we will investigate how CITK mutation or inactivation leads to DNA damage. Finally, we

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

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 strengthen our efforts to increase the collaborations between NICO and clinical researchers of the Department of Neuroscience. Specifically, we are working on the implementation at NICO of the genetically tractable model C. elegans, which will be of invaluable help in addressing the biological significance of mutations identified in a clinical setting, in patients affected by neurodevelopmental and neurodegenerative disorders.

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

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

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

Microcephaly

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

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

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

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

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

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

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

We found that CITK controls cytokinesis and spindle orientation by altering microtubule dynamics, a scenario supported by the results of our proteomics screen. These functions involve at least in part the capability of CITK to modulate TUBB3 phosphorylation through CK2a recruitment. Moreover KIF14, whose loss leads to microcephaly in mice and humans, is a partner of CITK in regulation of midbody stability. Since kinesins play

established roles in microtubule dynamics and CK2a has been involved in kinesins' regulation, we will set out to obtain more information about the interplay between all these molecules.

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

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

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

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

5. Identification of new genes involved in NDD.

We will work with our collaborators in the Genetics and Neuroscience departments 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 both transgenic and knockdown models in the genetically tractable model *C. elegans*. Moreover, we plan to use neural stem cell culture and also human forebrain organoids, derived from patient-specific induced pluripotent stem cells

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

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

f. Methodology (up to 2000 characters): <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 2022

Laboratory name: Neuropsychopharmacology

1. LABORATORY DESCRIPTION – PERSONNEL:[46]

Principal Investigator
Surname: Eva, name: Carola Eugenia, position: full professor, degree: PhD, birthdate: 21/07/1957, phone: +39 0116706608, email: carola.eva@unito.it

Personnel
Surname: Oberto, name: Alessandra, position: research associate (RU), degree: PhD, birthdate: 24/10/1967, phone: +39 0116706611, email: alessandra.oberto@unito.it, role & expertise: design, supervision and conduction of experiments with expertise in biotechnology, molecular and cellular biology, immunohistochemistry, behavioral analysis

Surname: Bertocchi, name: Ilaria, position: research assistant (RTD-A), degree: PhD, birthdate: 13/04/1982, phone: +39 0116706611, email: ilaria.bertocchi@unito.it, role & expertise: design, supervision and conduction of experiments with expertise in mouse behavior, molecular biology, immunohistochemistry

2. CURRENT GRANTS

Starting-end date	Project Title and ID	Beneficiary[47]	Funding Program/Agency	Role of the unit[48]	Overall Amount Funded	Managed by FCO/UNITO
2020-2022	Nuove prospettive terapeutiche nel trattamento della sindrome dell'X-fragile	Carola Eva	Fondazione CRT	PI	35000	UNITO

3. SCIENTIFIC ACTIVITIES IN 2022

Carola Eugenia Eva, (PI)

Supervised PhD students:	Giacomo Einaudi
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	-Daniela Carulli, Laboratory for Neuroregeneration Netherlands Institute for Neuroscience (NIN) Meibergdreef 47 - 1105 BA Amsterdam - The Netherland -Antonio Rodríguez-Moreno (Universidad Pablo de Olavide, Sevilla)

[46] For further personnel copy the corresponding form, and number accordingly; do not exceed one line to describe role & expertise
[47] 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.
[48] Coordinator/PI of research unit/team component.

	-Rolf Sprengel, Research Group of the Max Planck Institute for Medical Research at the Inst. for Anatomy and Cell Biology, Heidelberg University -Pierandrea Muglia, GRIN Therapeutics Inc, Brussels, Belgium
• Invited talks:[49]	
• Science communication:[50]	
• Editorial duties:	
• others[51]	Design and management of a study called 'Preclinical validation of a negative allosteric modulator of the NR2B-NMDA receptor, in the *Grin2a*$^{S/S}$ mouse model' at S&P BRAIN s.r.l and sponsored by GRIN Therapeutics, Inc
Organizational activities and responsibilities at NICO:	In charge for hygiene anti-smoke rules
Speakers invited:	
Other organizational activities:[52]	Founding member and President of the spinoff S&P BRAIN
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Alessandra Oberto, Research associate

Supervised PhD students:	Giacomo Einaudi
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	-Daniela Carulli, Laboratory for Neuroregeneration Netherlands Institute for Neuroscience (NIN) Meibergdreef 47 - 1105 BA Amsterdam - The Netherland -Mazahir Hasan, Ikerbasque Professor and Group Leader at the Achucarro Basque Center for Neuroscience heading the Laboratory of Brain Circuit Therapeutic -Rolf Sprengel, Research Group of the Max Planck Institute for Medical Research at the Inst. for Anatomy and Cell Biology, Heidelberg University -William Wisden, Imperial College London Dept Life Sciences Laboratory of Molecular Neuroscience
• Invited talks:	
• Science communication:	
• Editorial duties:	
• others	Conduction of experiments for a study called 'Preclinical validation of a negative allosteric modulator of the NR2B-NMDA receptor, in the *Grin2a*$^{S/S}$ mouse model' at S&P BRAIN s.r.l and sponsored by GRIN Therapeutics, Inc
Organizational activities and responsibilities at NICO:	In charge for behavioral labs I and II (animal facility)
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	

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

Technology transfer achievements (patents, etc.):	

Ilaria Bertocchi, Research assistant (RTD-A)

Supervised PhD students:	Giacomo Einaudi
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	-Mazahir Hasan, Ikerbasque Professor and Group Leader at the Achucarro Basque Center for Neuroscience heading the Laboratory of Brain Circuit Therapeutic -José María Delgado García, full Professor of Physiology Pablo de Olavide University Division of Neurosciences, Building 21, Pablo de Olavide -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 (United Kingdom) -Pierandrea Muglia, GRIN Therapeutics Inc, Brussels, Belgium -Daniela Carulli, Laboratory for Neuroregeneration Netherlands Institute for Neuroscience (NIN) Meibergdreef 47 - 1105 BA Amsterdam - The Netherland
• Invited talks:	'Modelli sperimentali per lo studio delle epilessie riflesse' 45° Congresso nazionale lega contro l'epilessia (LICE), Venerdì 10 Giugno 2022
• Science communication:	06/06/2022: 'I benefici del gioco di squadra'. Partecipazione a programma radiofonico Rai Radio 24, Obiettivo salute 22/12/2022: Partecipazione a 'Next cafè', Incontro organizzato da studenti della scuola secondaria di II grado 'Modificazione genetica e tecnica CRISPR-Cas9'
• Editorial duties:	Guest Associate Editor in Frontiers in Neuroscience- Gut-Brain Axis: 'Nutritional Modulation of Central Nervous System Development, Maintenance, Plasticity, and Recovery'. Topic Editors: Fausto Chiazza, Ilaria Bertocchi e Silvia Turroni. Topic Published: 07/10/2022
• others	NICO neurowebinar on 25/02/2022 'Role of perineuronal nets in fragile X syndrome' Principal investigator and responsible for conducting the study 'Preclinical validation of a negative allosteric modulator of the NR2B-NMDA receptor, in the *Grin2a*$^{S/S}$ mouse model' at S&P BRAIN s.r.l and sponsored by GRIN Therapeutics, Inc
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:	Dr. Shi-Bin Li, Stanford University (01/04/2022) 'Interrogation of sleep disorders associated with aging and stress'
Other organizational activities:	'The Science Bridge' advisory board member (https://thesciencebridge.org/) Comitato scientifico assmgrin2aitalia https://assmgrin2aitalia.it/comitato-scientifico/
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

ALL LAB MEMBERS

Activities:[53]	

4. Research activity in 2022[54]

a. Summary (500 characters)

We are investigating the effects of a ketogenic diet (KD) in a mouse model of a *Grin2a* syndrome. To this aim, we subjected the first cohorts of N2AM2S mice (males and females, homozygous and heterozygous for the mutation and wild-type as controls) to standard diet or KD for 5 weeks. After 3 weeks of diet mice underwent to a battery of behavioral tests for the evaluation of the cognitive and epileptic phenotype. Meanwhile, we are completing extensive work regarding the role of perineuronal nets (PNNs) in the pathogenesis of Fragile X syndrome (FXS).

b. Background and rationale (3000 characters)

1. *GRIN*-related disorders are neurodevelopmental disorders caused by rare variants and mutations in the N-methyl-D-aspartate receptor (NMDAR) subunit receptor *GRIN* genes. Patients present with a combination of symptoms of different degrees of severity largely including epilepsy, but also intellectual disability, autism, movement and behavioral disorders. The identification of effective antiseizure medications (ASM) remains a significant ongoing medical need for *GRIN*-related disorders, in particular for patients affected by the most severe pharmaco-resistant forms, namely developmental and epileptic encephalopathies (DEEs), largely associated to gain-of-function (GoF) mutations in the GRIN1, GRIN2B and GRIN2A genes.
   Our N2AM2S mice, carrying the GluN2A(N615S) mutation, are strongly prone to audiogenic generalized seizures (AGS) and show cognitive deficits and ADHD-like behavior when homozygous (*Grin2a*$^{S/S}$ mice), faithfully summarizing what occurs in patients with DEE harboring similar mutations, and thus representing a robust mouse model of *GRIN*-related DEEs (Bertocchi et al., 2021).
   High-fat, low-carbohydrate KD is an established and proven treatment for refractory epilepsy. Furthermore, several studies suggest that KD may enhance cognitive functions. However, only a few animal and human studies have investigated the effects of KD on cognitive impairment, and the results have not been conclusive. Thus, the aim of the research is to perform a preclinical study to evaluate the therapeutic efficacy of a KD in counteracting the cognitive and behavioral deficits and the epileptic seizures induced by the GluN2A(N615S) mutation.
2. Perineuronal nets (PNNs) are a particular mesh-like aggregation of extracellular matrix molecules which form around neuronal soma and proximal projections during postnatal development. Fast-spiking parvalbumin-positive GABAergic interneurons (PVIs) are the type of neurons preferentially enveloped in many cortical areas, and their coverage is considered a sign of maturity. The last decades have seen a gradual and increasing recognition of the remarkable role of PNN in controlling plasticity, and alterations of PNN expression and PVI density have been described in patients and animal models for various neuropsychiatric and neurodevelopmental disorders. In the Fragile X syndrome (FXS), the loss of FMRP activity provokes an upregulation of Matrix Metallopeptidases 9 (MMP-9) translation that leads to

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

increased PNN digestion. However, little is known about FXS-dependent alterations of PNN expression affecting activity and plasticity in various brain areas. Thus, we conducted an accurate study to systematically measure PNN and PVI distribution in the FXS mouse brain.

c. Objectives (1000 characters)

Objective 1: The main aim of the first research project is to study, for therapeutic purposes, the efficacy of new ASM (**A**) and, in particular, the impact of KD (**B**) on the physiology and behavior of a mouse model of *GRIN*-dependent DEE, in order to better understand the molecular mechanisms involved.

Objective 2: Considering that PNN alterations in various brain areas may be associated with characteristic FXS endophenotypes, the main objective of this year work was to conduct a thorough analysis of PNNs and PVIs to better correlate the presence of altered networks to specific behavioral deficits at different developmental stages, for possible future therapeutic approaches that has PNNs or MMP-9 as targets.

Results (4000 characters)

1- (**A**) We have conducted a study, commissioned to S&P BRAIN srl by the company GRIN THERAPEUTICS, Inc., to assess the therapeutic efficacy of a negative allosteric modulator of the NMDAR GluN2B subunit, in counteracting audiogenic seizures (AGS) in *Grin2a*$^{S/S}$ homozygous mice, our murine model of *GRIN2A*-related DEEs. The drug was acutely administered before the presentation of a high-frequency acoustic stimulus commonly used for AGS induction and proved to be significantly effective in dose-dependently protecting *Grin2a*$^{S/S}$ mice from this type of serious generalized seizures. Our data clearly suggest that the ongoing clinical trial with this new drug could also be extended to patients affected by syndromes associated with *GRIN2A* mutations and support its use as an effective and targeted ASM for *GRIN*-related disorders. The results of this work will be summarized in a paper, currently under preparation, to be submitted to a high-impact factor journal (Bertocchi et al, 2023) and presented at a symposium at the forthcoming 20th SINS national congress and the 37th ECNP European congress in 2024.

1- (**B**) We were able to collect an initial body of data regarding the performance of N2AM2S mice and wild-type littermates treated with KD or standard diet (SD) in a battery of behavioral tests. Mice were tested for: nesting and burrowing activity as innate behaviors; open field and rotarod to evaluate locomotor activity and motor functions; the CAR test for impulsivity and the rewarded T-maze and the Morris water maze to evaluate cognitive functions. We also evaluated the susceptibility to AGS before sacrifice. These analyses were conducted on consecutive days under diet regimen, which started at weaning (from P21 to P30), and the mice were sacrificed between P60 and P67. During the experimental protocol, the caloric intake and the body weight gain of the animals have been recorded weekly.

The resulting data are still being analyzed using a computerized video-tracking system (Ethovision XT video track system; Noldus Information Technology, Wageningen, The Netherlands). At the sacrifice, we collected several fresh tissues: blood, brains, fat, muscle, kidney, heart, liver and fecal samples for further molecular analysis.

Our preliminary results demonstrate that the KD is too stiff and heavy to be administered at P21 and therefore all the next cohorts will be tested starting at least at P30 and sacrificed only after 5 weeks of diet. Initial data suggest a beneficial effect of KD on AGS susceptibility in homozygous female mice. As far as other behaviors are concerned, it is still too early to say, because there are many experimental groups and the N number is still low.

2- We subjected an adequate, but not yet complete, number of mice at different stages of development [9-10 Fmr1KO and WT females and 8-8 Fmr1KO and WT males sacrificed to P20; 14 -13 Fmr1KO and WT females and 9-11 Fmr1KO and WT males sacrificed at P40;

and 9-6 Fmr1KO and WT females and 9-4 Fmr1KO and WT males sacrificed at P60] to a behavioral test battery and we are currently analyzing their brains for PNN and PVI distribution.
Thanks to our collaborations with internal NICO members (Prof Luzzati), we refined and validated a new customized method for brain-wide 3D reconstruction of histologically stained serial sections followed by high-throughput imaging analysis, to better characterize PNN and parvalbumin expression in the brain of Fmr1KO and control mice. This work is now completed and a manuscript is in preparation, which we plan to publish in a high impact factor scientific journal ('Whole brain parvalbumin and perineuronal net expression analysis in Fragile X mice' by Bertocchi et al). A similar accurate study has been recently conducted in adult C57BL/6 wild type mouse brains (Pizzorusso et al., 2023) and our data largely confirmed his findings. In addition to that, however, our work describes, for the first time, reduced level of PNNs, sometimes in conjunction with decrease PVI density, in different brain areas of FXS mouse brains. PNN reduction may be primarily attributed to the activity of MMP-9, but many other mechanisms can be involved.

d. Advancement in the field (1000 characters)

All studies mentioned above provide more insights into the molecular mechanisms of two serious neurodevelopmental pathologies: the developmental and epileptic encephalopathies (DEEs) and fragile X syndrome (FXS), and establish robust proof-of-concept for extending the use of drugs currently already in the clinic, but also for the discovery and preclinical evaluation of new targets and approaches, in order to meet the ongoing medical need for these rare pathologies.

e. Publications[55]

1- **Oberto A**, **Bertocchi I**, Longo A, Bonzano S, Paterlini S, Meda C, Della Torre S, Palanza P, Maggi A, **Eva C** (2022). Hypothalamic NPY-Y1R Interacts with Gonadal Hormones in Protecting Female Mice against Obesity and Neuroinflammation. Int J Mol Sci. 2022 Jun 6;23(11):6351. doi: 10.3390/ijms23116351. PMID: 35683029; PMCID: PMC9180984.

2- Alves GF, Stoppa I, Aimaretti E, Monge C, Mastrocola R, Porchietto E, **Einaudi G**, Collotta D, **Bertocchi I**, Boggio E, Gigliotti CL, Clemente N, Aragno M, Fernandes D, Cifani C, Thiemermann C, Dianzani C, Dianzani U, Collino M (2022). ICOS-Fc as innovative immunomodulatory approach to counteract inflammation and organ injury in sepsis. Front Immunol. 2022 Sep 2;13:992614. doi: 10.3389/fimmu.2022.992614. PMID: 36119089; PMCID: PMC9479331.

3- Nuzzo AM, Moretti L, Mele P, Todros T, **Eva C**, Rolfo A. (2022) Effect of Placenta-Derived Mesenchymal Stromal Cells Conditioned Media on an LPS-Induced Mouse Model of Preeclampsia. Int J Mol Sci. 2022 Jan 31;23(3):1674. doi: 10.3390/ijms23031674.

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.

[55] DO NOT include papers in press or submitted.

a. Summary (up to 2000 characters):

1. In the next future, we are planning to conclude our study investigating the effects of a ketogenic diet on N2AM2S mice (Bertocchi et al., 2021). In this way, we would like to reach a better understanding of the molecular mechanisms through which KD can alleviate seizures and cognitive deficits. This is fundamental for developing new drugs with an overall efficacy while avoiding side effects as much as possible. To do so we have established a collaboration with members of our department of neuroscience and with national and international experts in the field;
2. Thanks to the detailed whole-brain PNN and PVI analysis in FXS mice at different developmental stages, we would like to identify new molecular targets and the most effective time window for a proper therapeutic intervention. To do so, we have established a collaboration with NICO members and, again, with national and international experts in the field. Our desire is to carry on a highly innovative and attractive project that will allow us to compete in national and international calls for grants on rare diseases;
3. Thanks to our old mouse model with the floxed *Npy1r* gene, coding for the Y1 receptor for Neuropeptide Y (NPY), we have established a collaboration with the University of Bologna to investigate the role of the Y1 receptor in the inhibition of inflammation mediated by sympathetic nerve reflex activity;
4. Lastly, we are working with our longtime collaborator Prof. Hasan on active and parallel projects regarding the role of NMDA receptors in cognitive functions, and we currently have 2 papers under review (iScience and Molecular Psychiatry) and one project about astrocyte-NMDAR-dependent gliotransmission in progress.

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

1- Despite the demonstrated utility in some forms of DEE, the mechanisms of the ketogenic diet in epilepsy are not fully understood. It has been proposed that it favorably affects brain metabolism by increasing energy stores along with increased synthesis of GABA, which leads to greater resistance to seizures in ketotic brain tissue. In addition, this particular therapeutic diet has also shown beneficial effects on cognitive functions. However, only few interventional studies in animals and humans have addressed the effects of KD on cognitive impairments, and the results were inconclusive. It is a dietary therapy that is difficult to adhere to and is not suitable for all patients; it is important to exclude metabolic problems that may be aggravated by the diet and requires strict adherence to quantities and doses; it is expensive, and continuous monitoring is necessary, with the need for concomitant administration of vitamin-mineral supplements, in addition to monitoring the level of ketosis and frequent side effects. For these reasons, despite its documented effectiveness, it is not well accepted and difficult to implement. A combination of genetic, environmental and biological factors (including metabolic and neuronal inflammation) contributes to the variability in seizures susceptibility and cognitive and physiological impairments observed in patients affected by DEE syndromes. A better understanding of the molecular mechanisms through which KD is able to improve the susceptibility to seizures and cognitive deficits is fundamental for the possible development of new drugs, which facilitate the achievement of the same beneficial results induced by this therapeutic regimen, but without the problem of compliance and side effects induced by its chronic intake.

2- The last decades have seen a gradual and increasing recognition of the remarkable role of PNN in controlling plasticity, and alterations of PNN expression and PVI density have been described in patients and animal models for various neuropsychiatric and neurodevelopmental disorders. No approved therapies exist for FXS, but several potential medications targeting

different pathways involved in FXS pathophysiology can be beneficial in patients depending on the individual constellation of comorbid symptoms. As PNNs may play a major role in FXS pathology, the development of therapeutic interventions which target PNNs may be key in reversing several FXS phenotypes, including behavioral deficits and neuronal hyperexcitability. Animal models are well suited for this purpose; they offer the advantage of studying pathways relevant to the disease pathology, as often the principal target is not druggable.

3- Through the activation of the inflammatory reflex, the nervous system has the ability to inhibit inflammation: the afferent arm of the reflex starts from the periphery and signals to specific areas of the CNS the existence of an ongoing immune challenge. Once the information has arrived, the CNS processes it and activates the efferent arm of the reflex: this is composed of the sympathetic splanchnic nerves and is defined as the splanchnic anti-inflammatory pathway (SAIP) (Martelli et al., 2014). The aim of the study is to investigate the possible role of two candidate receptors, the β2ADRs and the NPY1Rs, in mediating the effects of the efferent arm of the inflammatory reflex. This nerve reflex inhibits the development of an exaggerated inflammatory response.
4- The primary motor cortex (M1) plays an essential role in instrumental and classical conditioning, requiring NMDA receptor-dependent neuronal plasticity. It is, however, not known what role the M1 astrocyte NMDA receptor plays in associative learning. We hypothesized that astrocyte-NMDAR-dependent gliotransmission might control learning through intimate communication with neuronal circuits. We removed the NMDA receptor selectively in astrocytes of the M1 cortex to investigate this question.

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

Our projects fit very well with the mission of the Institute, because their intention is to gain more knowledge about rare genetic disorders affecting young children with really debilitating symptoms. Considering that current antiseizure medications (ASMs) are often unable to alleviate the major comorbid features associated, including psychiatric problems such as ASD-like and ADHD-like behaviors, and lead to important side effects, there is a great need to search for new treatments for seizures and their comorbidities. However, this process faces considerable challenges.

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

1. '*Effects of the ketogenic diet in a mouse model of GRIN-related developmental and epileptic encephalopathy'*
   The main objective is to perform a preclinical study of the therapeutic efficacy of the KD in counteracting AGS and cognitive and behavioral deficits induced by the GluN2A(N615S) mutation in mice. We aim to obtain a significant number of mice for each experimental group, that are 12: male and females mice belonging to 3 different genotypes (homozygous and heterozygous for the mutation and their wild-type littermates) under 2 different diet regimens (KD and SD). Different cohorts of mice, obtained by crossing heterozygous mutant mice, will be subjected to the diet at the time of weaning (around P30) for 5 weeks. Mice will be subjected to the diet and a battery of behavioral tests as described previously. After having evaluated the efficacy of KD on cognitive development and on AGS of mutant mice we will analyse the expression of marker of neuronal plasticity and of neuroinflammation by histochemistry on brain slices. In collaboration with Prof Collino laboratory, the systemic glycidic and lipid profiles will be analyzed on the blood collected, as well as markers indicative of liver function and of metabolic inflammation. Samples of liver and skeletal muscle, appropriately collected *post mortem* and stored at -80C, will be processed by molecular biology techniques and used to evaluate the impact of KD on the development of steatosis and on the expression of regulatory molecules (i) of processes of gluconeogenesis and insulin

resistance, (ii) of lipid metabolism and (iii) of the development of a local inflammatory response (with particular reference to the study of the impact of KD on the activation of the inflammatory protein complex NLRP3 inflammasome).

2. *'Perineuronal nets: a new therapeutic target for fragile X syndrome'*
   Our study will allow to define with reliability and precision the brain areas to investigate in our preclinical study for testing drugs known to modify the expression of PNNs. The analysis will be focused on the behavioral, molecular and physiological parameters that were shown to be altered in Fmr1KO, compared to WT mice. We will also analyze the intrinsic neuronal excitability, glutamatergic and GABAergic synaptic transmission in brain slices by patch-clamp recordings thanks to the collaboration with Prof. Tempia group. Postsynaptic proteins with altered expression will be analyzed by WB by collaborators in Rome, where parallel analyses will be conducted in two distinct laboratories. In the first one (Prof Chiurazzi, geneticist), components of the ECM/PNNs and the efficacy of pharmacological treatments will be checked on cells derived from FXS patients (fibroblasts and iPS-derived neurons). In the second one (Prof. Altieri, biochemist), a virtual screen followed by *in vitro* analysis will be conducted, aimed to identify new MMP-9 inhibitors that may facilitate the design of future clinical candidates for FXS treatment.

4- *'The receptor implicated in the inhibition of inflammation mediated by sympathetic nerve reflex activity'*
The experimentation will be carried out in the laboratories of Prof. Martelli in Bologna where, thanks to our knock out mice for Y1R, he will be able to test the scientific hypothesis that the sympathetic nerves inhibit inflammation induced by a systemic immune challenge by releasing norepinephrine (NA) and neuropeptide Y (NPY) by binding to β2-adrenergic receptors (β2ADRs) and NPY1 receptors (NPY1Rs), respectively. Mice knockout for β2ADRs will be provided by Heidelberg University through our collaborations.

5- *'Learning after cortical astrocyte NMDAR removal'*
In our previous study (Hasan et al., 2013), we deleted the *Grin1* gene that encodes for the obligatory subunit of NMDARs in the primary motor cortex and found that it is needed for synaptic plasticity and trace eyeblink conditioning, a prototypical model of declarative memory. We wondered about the putative role of astrocyte NMDARs in the modulation of synaptic activity and learning and memory processes. Based on previous finding where blocking hippocampus gliotransmission impaired NMDAR-dependent synaptic plasticity, we hypothesized that astrocyte-NMDAR might activate gliotransmission, possibly at the tripartite synapse, to modulate synaptic plasticity and learning and memory processes. To investigate such hypothesis, thanks to the floxed *Grin1* mice present in our facility, we will generate astrocyte-specific *Grin1* gene knockout mice by using rAAVs for Cre recombinase-dependent gene deletion under the GFAP promoter.

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

Our research is based on the use of different murine models, which are unique and precious tools to identify new molecular targets, and essential to provide prospects for effective therapeutics to treat various neurodisorders. The search for such effective treatments is a priority for science and society, in particular for rare diseases for which there is still no cure.
Moreover, our work on memory engram using the pre- and postsynaptic NMDAR plasticity and interrogating the role of specific astrocyte expressed NMDARs, is fundamentally important to understand the systems consolidation mechanism, from molecular to circuit levels, and provides enormous possibilities to apply the genetic technologies developed by our collaborator and used in our studies to reveal fundamental insight into different neuropsychiatric disorders in the future.

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 2022

Laboratory name: Neuroendocrinology

1 LABORATORY DESCRIPTION – PERSONNEL:[56]

Principal Investigator (Acting Group leader)
Surname Gotti, name: Stefano, position: Associate Professor, degree: PhD, birthdate: 17/06/1971, phone: 0116706610, email: stefano.gotti@unito.it

Personnel

1) Surname: Marraudino, Name: Marilena, position: Fondazione Veronesi fellow, degree: PhD, birthdate: 08/06/1988, phone: 0116706632, email: marilena.marraudino@unito.it,
   Role & expertise: Researcher; Control of reproduction, endocrine disruptors

2) Surname: Bonaldo, name: Brigitta, position: academic Research fellow, degree: PhD, birthdate: 30/01/1992, phone: 0116706632, email: brigitta.bonaldo@unito.it,
   Role & expertise: Researcher; Neurodegenerative disorders models, endocrine disruptors

3) Surname: Morgan, name: Stephen, position: PhD-student, degree: Master Degree, birthdate: 27/06/1993, phone: 0116706632, email: godstime.morgan272@edu.unito.it,
   role & expertise: Researcher; eating disorders models

4) Surname: Casile, name: Antonino, position: PhD-student, degree: Master Degree, birthdate: 26/04/1991, phone: 0116706632, email: antonino.casile@unicam.it,
   role & expertise: Researcher; eating and gaming disorders models

5) Surname: Ballan, name: Chiara, position: PhD-student, degree: Master Degree, birthdate: 20/09/1995, phone: 0116706632, email: chiara.ballan@edu.unito.it,
   role & expertise: Researcher; endocrine disruptors

2 CURRENT GRANTS

Starting-end date	Project Title and ID	Beneficiary[57]	Funding Program/Agency	Role of the unit[58]	Overall Amount Funded	Managed by FCO/UNITO
2019-2022	Dipendenze dalle nuove tecnologie: sviluppo di un modello animale per future applicazioni sull'uomo	Panzica, PI	Fondazione CRT	Coordinator	30000	FCO
2020-2022	Ruolo degli ormoni steroidei nella patogenesi e nello sviluppo dei tumori gliali: nuove frontiere per	Panzica, PI	UNITO-Dipartimento Progetti traslazionali	Co-PI (other Co-PI Prof. Garbossa)	15000	UNITO

[56] For further personnel copy the corresponding form, and number accordingly; do not exceed one line to describe role & expertise
[57] 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.
[58] Coordinator/PI of research unit/team component.

	approcci terapeutici					
2019-2022		Panzica-Gotti, PI	UNITO-autofinanziata	Coordinator	13648	UNITO
2020-2023	La deprivazione affettiva nell'Anoressia Nervosa: possibile ruolo dell'Ossitocina; studio sul modello animale ABA.	Gotti, PI	Fondazione CRT	Coordinator	35000	UNITO
2021-2023	SARS-CoV-2 e non solo: portare fino alla sperimentazione umana un candidato farmaco antivirale pan-coronavirus. *Thinking innovative to fight the unexpected*.	Gotti, PI	Regione Piemonte/FIN Piemonte	PI of research unit	80799	UNITO
2021-2024	Developmental, Reproductive and Metabolic effects of Endocrine Disruptors: the DReaM-ED study	Gotti, PI	PRIN 2020	PI of research unit	134696	UNITO
2022-2024	Soy: a good nutritional supplement in Anorexia Nervosa during pregnancy on the Health of Mothers and Offspring?	Marraudino	Brain and Behavior Research Foundation	PI of research unit	69753 $	FCO

3 SCIENTIFIC ACTIVITIES IN 2022

Stefano Gotti (PI)

Supervised PhD students:	Brigitta Bonaldo (co-supervisor with GianCarlo Panzica), Godstime Stephen K. Morgan, Chiara Ballan
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	Cooperation with dr. P. Collado (UNED, Madrid, Spain) Cooperation with D. Grassi (Universidad Autonoma, Madrid, Spain)
• Invited talks:[59]	

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

• Science communication:[60]	
• Editorial duties:	Guest editor in the Special Issue "Metabolism Disrupting Chemicals: Understanding Their Role in Metabolic Disease 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[61]	
Organizational activities and responsibilities at NICO:	First aid and fire safety officer
Speakers invited:	
Other organizational activities:[62]	
Workshops, Schools or Conferences organized:	Member of the Educational Committee of the International Meeting Steroids and Nervous System and Organizer of the Virtual Meeting Steroids and Nervous System, February 2022
Technology transfer achievements (patents, etc.):	

ALL LAB MEMBERS

Activities:[63]	**Marraudino**. Ricercatore in classe. Dissemination meetings for 'Researcher in the classroom' project of the Umberto Veronesi Foundation aimed at high schools: 27/05/2022, Liceo Alfieri, Torino. 20/04/22, Liceo Umberto I, Torino. **2/6/2022 - TRMh24 news interview.** 'Siamo ciò che mangiamo: le scoperte sulla soia della ricercatrice materana Marilena Marraudino'. **1/10/2022 - U*NIGHT - La Notte Europea dei Ricercatori e delle Ricercatrici 2022.** The Green Brain: un "Caffe Scientifico" per comprendere l'impatto di alimenti e inquinanti ambientali sulla salute del nostro cervello. 22/11/2022 - **Workshop 'Fisica: nome comune genere femminile una scelta senza stereotipi'**. Dipartimento di Fisica, Università di Torino. Seminario dal titolo: 'L'encefalo dei due sessi, uno strano mosaico'.

4. Research activity in 2022[64]

a Summary (500 characters)

[60] Public engagement
[61] Posters at meetings, participation in the board of scientific societies, referee for grant agencies
[62] No university appointments.
[63] List here activities where all member participated or group activities to avoid duplications (eg Open days at NICO). Add lines when needed.
[64] Use times new roman 11 for the text.

In 2022 we mainly focused on two research topics: the study of the effect of exposure to Endocrine Disruptors towards nervous circuits and the behavioral and morphological study of a rodent model of Anorexia Nervosa.

b Background and rationale (3000 characters)

Steroid hormones exert a large array of biological effects on the nervous system: a lot of nuclear estrogen receptors (ERα and ER β) and membrane receptor (GPER-1) are expressed in many brain areas during ontogeny, and estrogens may modulate neuronal differentiation, notably by influencing cell migration, survival and death, and synaptic plasticity. Appropriate levels of gonadal hormones are therefore 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. Our studies therefore are focused on understanding what happens when this delicate hormonal balance is disturbed by "external" factors. For this reason, we performed experiment that mimic an alteration of the environment to observe the possible influence in the neural circuits.

One research line involves the study of substances collectively named Endocrine Disrupting Chemicals (EDCs), that can alter the functions of the endocrine system which is intimately connected to the development and functioning of the nervous system. Different EDCs are xenoestrogens or xenoandrogens, and they could deeply influence the development and the function of gonadal hormones-dependent neural circuits and related behaviors. 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.

Another research line involves the effect of early stressors like maternal separation and eating disorders in the alteration of different neuronal circuits. Among eating disorders, Anorexia Nervosa (AN) is one of the most severe types, recording the highest mortality rate of any psychiatric disorder. AN is a complex and multifactorial disorder resulting from environmental stressors as well as genetic factors, and typically affects women. Several animal models have been devised to simulate replicable biological correlates and underline the pathophysiological condition in AN patients. One of the most studied is the activity-based anorexia (ABA): a model for self-starvation in which animals exposed to a restricted feeding scheme and unlimited access to a running wheel undergo to a steady weight loss; ABA model have a significant impact on energy metabolism, reward circuitry, and bone physiology, alterations present in about 80% of AN patients.

c Objectives (1000 characters)

Our main goal was double:

Regarding EDCs: the study of the interactions among EDCs and neural circuits-behavior. In particular, we analyzed the effect of perinatal exposure to different EDCs. We focalized our attention to neuroendocrine circuits controlling feeding behavior, maternal behavior, and reproduction.

Regarding AN: we analyzed the influence of early maternal separation on susceptibility of ABA model.

d Results (4000 characters)

Endocrine Disruptors Compounds (EDCs) effects in the nervous system

Exposure to EDCs is especially dangerous if it occurs during specific “critical periods” of life, when organisms are more sensitive to hormonal changes.

In one study (**1**) we focused on the effects of perinatal exposure to an organotin such as tributyltin chloride (TBT), a highly diffused environmental pollutant which act as metabolism disrupting chemicals; TBT may interfere with fat tissue differentiation, as well as with neuroendocrine circuits, thus impairing the control of energetic balance. We orally administered daily different concentration

of TBT to pregnant female mice from gestational day 8 until birth, and to their pups from day 0 until post-natal day 21. Our results showed that TBT exposure of female mice during gestation and of pups during lactation permanently altered the feeding efficiency of pups of both sexes and subcutaneous fat distribution in adult males. In addition, the neuropeptide Y system was affected.
Another study (**2**) is focused on the exposure to either bisphenol A or S (BPA, BPS) as a risk for crucial behaviors for pup survival, such as spontaneous maternal behavior in mice. BPA is known to exert negative effects on maternal behavior. BPS, a BPA-substitute, seems to share some endocrine disrupting properties. We analyzed the effects of low-dose BPA or BPS exposure throughout pregnancy and lactation in mice. During the first postnatal week we observed the spontaneous maternal behavior. At the pups' weaning, we sacrificed the dams and analyzed the oxytocin system, involved in the control of the maternal care, in the hypothalamic magnocellular nuclei. At birth, pups from BPA-treated dams tended to have lower male-to-female ratio compared to controls, while the opposite was observed among BPS litters. During the first postnatal week, offspring mortality impacted differentially BPA and BPS litters, with more female dead pups among the BPA litters, while more male dead pups in the BPS litters, sharpening the difference in the sex ratio. BPA and BPS dams spent significantly less time in pup-related behaviors than controls. Oxytocin-ir in paraventricular and supraoptic nuclei was increased only in the BPA-treated dams.

Translational models of Anorexia Nervosa
A principal animal paradigm employed in Anorexia Nervosa (AN) study is the activity-based anorexia (ABA) model. The model's efficacy in recapitulating the core features of AN in humans allows for the study of the parameters involved in the disorder. Our study (**3**) examined the susceptibility to the ABA protocol in the presence of a significant stressor (maternal separation) in male and female rats. More importantly, we analyzed the sex-differences on activity levels during different periods of the ABA protocol to determine the period(s) influencing the most pathological weight loss. This study aids in understanding the effect of intensity of activity during specific periods on the pathological weight loss.

PVN and Estradiol
Estradiol and hypothalamic paraventricular nucleus (PVN) help coordinate reproduction with body physiology, growth, and metabolism. PVN integrates hormonal and neural signals originating in the periphery, generating an output mediated both by its long-distance neuronal projections, and by a variety of neurohormones produced by its magnocellular and parvocellular neurosecretory cells. We review (**4**) the cyto-and chemo-architecture, the connectivity and function of PVN and the sex-specific regulation exerted by estradiol on PVN neurons and on the expression of neurotransmitters, neuromodulators, neuropeptides and neurohormones in PVN.

Sexual Differences in Gaming Disorder (GD)
We recently initiated a project regarding the creation of an animal model of GD, that it has been included by the WHO as a mental disorder. GD has different characteristics in the two sexes and is more prevalent in males. However, even if the female gamer population is constantly growing, most available studies analyzed only males. To better elucidate sex differences in GD, we selectively reviewed (**5**) research publications that evaluated GD separately for males and females collected in approximately one hundred publications over the past 20 years. The available data indicate that GD is strongly dimorphic by sex for both its psychological features and the involvement of different brain areas.

e Advancement in the field (1000 characters)

Our findings confirm that EDCs may have a dramatical effect in pups exposed during the perinatal periods of life; we observed different alterations in males and females in various area of the brain.

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.

a. Publications[65]

1. Ponti G, Bo E, Bonaldo B, Farinetti A, Marraudino M, Panzica GC, Gotti S. 2022 Perinatal exposure to tributyltin affects feeding behavior and expression of hypothalamic neuropeptide Y in the paraventricular nucleus of adult mice. Journal of Anatomy. Feb;242(2):235-244.
2. Bonaldo B, Gioisa L, Panzica GC, Marraudino M. 2022 Exposure to either bisphenol A or S represents a risk for crucial behaviors for pup survival, such as spontaneous maternal behavior in mice. Neuroendocrinology. Jul doi: 10.1159/000526074.
3. Morgan GSK, Mata Y, Carrillo B, Pellón R, Collado P, Gotti S, Pinos H. 2022 Influence of early maternal separation on susceptibility to the activity-based anorexia model in male and female Sprague Dawley rats. Neurosci Res. 2022 Nov;184:54-61. doi: 10.1016/j.neures.2022.08.001. Epub 2022 Aug 8. PMID: 35948154
4. Grassi D, Marraudino M, Garcia-Segura LM, Panzica GC. 2022 The hypothalamic paraventricular nucleus as a central hub for the estrogenic modulation of neuroendocrine function and behavior. Frontiers in Neuroendocrinology. 2022 Jan 5;65:100974.
5. Marraudino M, Bonaldo B, Vitiello B, Bergui GC, Panzica GC. 2022 Sexual Differences in Internet Gaming Disorder (IGD): From Psychological Features to Neuroanatomical Networks. J. Clin. Med. Feb 16;11(4):1018.

5. 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 - Effects of perinatal exposure to bisphenols (BPA or BPS) in mice pups' survivals and in different hypothalamic circuits, and in anxiety-like/anxiety-related behaviors.

2 - Effects of Genistein on neural circuits controlling reproduction, metabolism, and other physiological parameters.

3 - Effects of Estrogens treatment on neural circuits controlling reproduction and metabolism.

4 - Effects of hormones in the development of a rodent model of Anorexia Nervosa.

5 - Effects of hormones in the development of a rodent model of Gaming Disorder.

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

Steroid hormones, synthesized from cholesterol, are produced in various organs like the adrenal glands, gonads, or placenta, and exert several biological effects: these hormones, in fact, play important roles in the development, growth, maturation, differentiation and protection of the central and peripheral nervous system. Moreover, 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.

[65] DO NOT include papers in press or submitted.

Steroid hormones produced by gonads are implicated in the development of sexually dimorphic circuits and functions, in the control of physiological activities as reproduction, metabolism, parental behavior, social behaviors, and aggressive behavior. 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.
Our works are 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, in a broad sense, may exert a great impact on neural circuits; in fact, 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.
What is the main problem of the EDCs? These environmental contaminants have endocrine activity in humans, as well as in wildlife and domestic animal species. Some "natural" EDCs, i.e., 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. More recently, was develop the concept of metabolic disruptors: substances that can induce profound alterations of the metabolism. 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 major aim is to understand how the steroid hormones may interact and regulate the neural circuits that are involved in the control of important physiological activities (i.e., reproduction, food intake, metabolism), with consideration of gender differences.
This purpose is closely related to the 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. Moreover, 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 plan to continue our projects that are focused on the study of neuroendocrine system, neurogenerative and psychiatric diseases and their possible relation with environmental factors.

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

We will focus towards two main topics:

- **Effect of Hormones and EDCs**

Bisphenols effects.
We will investigate the effects of BPA and BPS treatment in different age of life: first, we want to analyze the effect of a perinatal exposure to BPA or BPS in a rodent model of multiple sclerosis. Considering the increasing exposure to EDCs and that the environmental components have been

implicated in the etiology of Multiple Sclerosis, this study aimed to better understand the consequences of perinatal exposure to BPA and BPS, in EAE-affected mice of both sexes.
Secondly, considering that exposure to bisphenols can lead to a wide range of effects, focusing on the effects of perinatal exposure to BPA or BPS, we will investigate the effects on anxiety-related behavior and the Raphe population of serotonin neurons involved in controlling this behavior. And finally, we want also to study the effects on the hypothalamic kisspeptin system and reproductive behaviors in mice.

Genistein (GEN) effects in pups.

GEN, a phytoestrogen widely found in soybeans, exhibits estrogen-like activity, acting as an endocrine disruptor that is particularly dangerous when administered during development at specific "critical" periods, such as postnatal age. Male and female CD1 mice will treated orally with GEN or vehicle alone during the first 8 days of life (PND1-PND8). At the age of 60 days, the animals will test for anxiety behavior and then sacrificed to analyze the circuit involved in anxiety behavior

Estrogens effects in pups.

Many hypothalamic systems controlling metabolism reproduction are programmed and stabilized during critical periods of development by many factors, including gonadal steroids. Estradiol (E_2) appears to have an important role in the organization of these circuits. E_2 acts through three different receptors: ERα, ERβ and GPR30. To understand the role of these receptors on organizational effect of E_2, we will treat male and female CD1 mice from post-natal day (PND) 5 to PND12 with subcutaneous injections of vehicle, E_2 and E_2 associated with selective antagonist of estrogen receptors (MPP; PHTPP; G15) alone or together (mix).

- **Translational studies**

Anorexia Nervosa and Activity-based anorexia (ABA) model.

Using a mild version of the ABA model we want to analyze the role of gonadal hormones in the sexually dimorphic effects of combined stressors, represented by early-life stress (*i.e.*, maternal separation, MS) and the induction of Activity-Based Anorexia (ABA) in juvenile rats. Thus, we will investigate the behavioral and neural alterations of gonadectomized rats of both sexes subjected to both MS and ABA.

Rat model of gaming disorder (GD)

Gaming disorder (GD) is classified as a mental disorder and has different characteristics in the two sexes and is more prevalent in males than females. More research is needed to better understand sex differences in GD and animal models could help to elucidate the neurological basis of this disorder. We want to develop and validate a rat animal model for studying the Gaming Disorder (GD). We are interested in sex differences in addictive behavior and brain activity during play and in possible alteration in several circuits involved in reward system.

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

Our research unit has always been interested in the interactions among steroid hormones and the nervous tissue, using as main physiological endpoint the behavior. In several brain areas are present a lot of steroid hormone receptors, and it is known that steroid hormones are involved in neuronal and glial differentiation, survival, and protection; thus, we think that a better understanding of the relationships among steroids hormones and nervous system is important. This interaction can partly explain gender differences in both physiological and pathological conditions.
Additionally, 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 many synthetic substances may interact with hormone receptors and therefore induce endocrine unbalance and diseases.
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 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 2022

Laboratory name: Peripheral Nerve Regeneration Unit

1 LABORATORY DESCRIPTION – PERSONNEL:[66]

Principal Investigator

Raimondo Stefania

Associate Professor

PhD in Physiology

Birthdate: 25/02/1977

Phone: +39 011/6705433

email: stefania.raimondo@unito.it

Personnel

Ronchi Giulia

Position: Associate Professor

PhD in Neuroscience, Master's degree in Neurobiology, University of Turin

Birthdate: 27/11/1982

Phone: +39 011/6705433

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

Gambarotta Giovanna

Position: Associate Professor (from March, 1, 2022)

Degree: PhD in Cellular Sciences and Technologies,

Master's degree in Biological Sciences, University of Turin

Birthdate: 22/08/1967

Phone: +39 011/6705436

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

Fregnan Federica

Position: Research Technician

Degree: PhD in Neuroscience

Master's degree in Biological Sciences, University of Turin

Birthdate: 02/07/1976

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

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

Muratori Luisa

Position: Research assistant (from 01/07/2022)

Degree: PhD in Experimental Medicine and Therapy

Master's Degree in Neurobiology

Birthdate: 02/05/1984

Phone: +39 011/6703577

Email: luisa.muratori@unito.it

Role & expertise: In vitro and in vivo models for autonomic nervous system regeneration

Fornasari Benedetta (concluded April 2022)

Position: Post-doctoral fellowship recipient

Degree: PhD in Neuroscience

Master degree in Molecular and Cellular Biology, University of Turin

Birthdate: 11/07/1989

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

El Soury Marwa (from 01/12/2022)

Position: Post-doctoral fellowship recipient

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

Birthdate:22/04/1991

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Role & expertise: Biomolecular analysis of peripheral nerve regeneration

Crosio Alessandro

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

Degree: Master degree Medicine and surgery, University of Torino

Birthdate: 04/08/1987

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

Zen Federica

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

Degree: Master degree in Industrial Biotechnologies, University of Padova

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Role & expertise: Biomolecular analysis of peripheral nerve regeneration

Garcia Bejarano Marina

Position: PhD student, PhD in Neuroscience

Degree: Master degree in Tissue Engeneering and Advanced Therapies, University of Granada

Birthdate: 14/10/1996

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Role & expertise: Biomolecular analysis of peripheral nerve regeneration

2 CURRENT GRANTS

Starting-end date	Project Title and ID	Beneficiary[67]	Funding Program/Agency	Role of the unit[68]	Overall Amount Funded	Managed by FCO/UNITO
12/2019-04/2023	Ricerca Finalizzata 2018	Prof.ssa Giovanna Gambarotta	Regione Piemonte	Member of the Research Unit	56.500	UNITO
01/2022-12/2023	Ex-post di progetti di ricerca di Ateneo	Prof.ssa Giovanna Gambarotta	Compagnia di San Paolo	PI	51560	UNITO
07/2022-12/2023	Grant for Internationalization	Prof.ssa Giovanna Gambarotta	Ateneo Torino	PI	11000	UNITO
07/2022-12/2023	Grant for Internationalization	Prof.ssa Giulia Ronchi	Ateneo Torino	PI	15233	UNITO
07/2022-12/2023	Grant for Internationalization	Prof.ssa Stefania Raimondo	Ateneo Torino	PI	22666	UNITO

3 SCIENTIFIC ACTIVITIES IN 2022

Stefania Raimondo (PI)

Supervised PhD students:	Alessandro Crosio (PhD) Federica Zen (PhD)

[67] 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.

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

	Monica Maurina (MD/PhD)
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	University of Granada, Spain, prof. Victor Sebastian Carriel University of Hannover, Germany, prof Kirsten Haastert-Talini University of Zaragoza, Spain, prof Jesus Ciriza University of Porto, Portugal, prof. Ana Colette Maurício University of Trás-os-Montes and Alto Douro, Vila Real, Portugal, dott. Artur Varejão Kyushu Institute of Technology, Fukuoka, Japan, prof. Yuki Shirosaki
• Invited talks:[69]	Virtual conference BIOMATMEET2022 "Biomaterials and Tissue Engineering in Peripheral Nerve Repair" APRIL 18-19, 2022
• Science communication:[70]	Notte dei Ricercatori 2022
• Editorial duties:	Editorial Board Member of *Frontiers in Neuroanatomy* Editorial Board Member of *Biomedicines* Guest Associate Editor for *Frontiers in Cellular Neuroscience* Guest Associate Editor for the special issue "Advance Research in Peripheral Nerve Regeneration" for the journal Biomedicines. Referee for Bioengineering, Neural Regeneration Research (NRR), Experimental neurology, Frontiers in Cellular Neuroscience
• others[71]	Referee for grant agencies: Swiss National Science Foundation. Board member of ESPNR (The European Society for the Study of Peripheral Nerve Repair and Regeneration) Member of "NANBIOSIS Scientific Advisory Board", Spain
Organizational activities and responsibilities at NICO:	NA
Speakers invited:	NA
Other organizational activities:[72]	NA
Workshops, Schools or Conferences organized:	Member of the scientific organizing committee for the SIBS congress, Torino 2022.
Technology transfer achievements (patents, etc.):	NA

Giulia Ronchi, Associate Professor

Supervised PhD students:	Alessandro Crosio (PhD) Monica Maurina (MD/PhD) Federica Zen (PhD) Riccardo Aucello (PhD)
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	University of Hannover, prof Kirsten Haastert-Talini University of Porto, Portugal, prof. Ana Colette Maurício

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

• Invited talks:	N.A.
• Science communication:	Notte dei ricercatori 2022
• Editorial duties:	Editorial Board Member of *Micro* (Microscale Biology and Medicines Section) Youth Editorial Board Member di *Neural Regeneration Research* (NRR) Referee for Biomedicine, Cells, Molecular Neurobiology, NRR and others
• others	Reviewer for "FWF" (Austrian Science Fund)
Organizational activities and responsibilities at NICO:	N.A.
Speakers invited:	N.A.
Other organizational activities:	N.A.
Workshops, Schools or Conferences organized:	Member of the scientific organizing committee for the SIBS congress, Torino 2022.
Technology transfer achievements (patents, etc.):	N.A.

Giovanna Gambarotta, Associate Professor

Supervised PhD students:	Federica Zen (PhD) Marina Garcia Bejarano (PhD)
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	University of Zaragoza, prof Jesus Martinez de la Fuente University of Granada, prof. Victor Sebastian Carriel University of Hannover, prof Kirsten Haastert-Talini
• Invited talks:	Stark Neurosciences Research Institute, Indiana University School of Medicine, 22/02/2022 "Cells & factors involved in the regeneration of peripheral nerves".
• Science communication:	Notte dei ricercatori 2022
• Editorial duties:	Member of Editorial Board of Brain Sciences Guest Associate Editor for Frontiers in Cellular Neuroscience Referee for *Biomolecules*, *Frontiers*, Brain *Sciences*, and others.
• Others	
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.):	Geuna S., Shahar A., Ziv-Polat O., Gambarotta G., Fregnan F. Potenziamento dell'effetto di Neuregulina1 di tipoI beta 1 sulla rigenerazione del nervo periferico mediante coniugazione covalente a nanoparticelle di ferro. Novembre 11, 2015 (domanda n° ITUB20155471).

ALL LAB MEMBERS

Activities:[73]	

4. Research activity in 2022[74]

a. Summary (500 characters)

The research activities of the group have been focused on the study of peripheral nerve (PN) repair and regeneration. In 2022 different research topics have been studied: i) understanding the biomolecular and biological processes occurring during PN regeneration, ii) methodology and techniques to repair severe somatic and autonomic injury with nerve substance loss, iii) evaluation of microbiota alteration on peripheral nervous system.

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,v) the loss of the Schwann cell (SC) capability to support regeneration, vi) the patient's age, vii) comorbidities.
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 follow 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.

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

c. Objectives (1000 characters)

The objectives of the group activities were to further understand the biomolecular and biological processes involved in nerve regeneration and to study how to improve 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 using different conduits or the effect of microbiota on the peripheral nervous system; 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/nerve transfer) to improve the regeneration of the peri-prostatic nerve after radical prostatectomy.

d. Results (4000 characters)

Chitosan membrane to improve cavernous nerve regeneration
Radical prostatectomy (RP) for prostatic cancer resections results in erectile dysfunction due to damage of the peri-prostatic nerve bundles.
In the current year, flat and nanostructured membranes with two different topographies, grating arrangement and zig-zag pattern were applied in vivo to repair cavernous nerve injury on adult male rats. Morphological analysis of the whole membranes harvested 60 days after the surgical procedure allowed to detect pathway of regenerated fibers on the devices. Results provide the first experimental evidence supporting the ability of the chitosan membrane to allow autonomic axonal regeneration in vivo demonstrating the safety of the device for clinical use and supporting its application as an effective adjunct strategy to reach the functional recovery after RP.

Somatic to autonomic: sciatic nerve transfer to penis corpora cavernosa for erectile dysfunction treatment
In patients that have been undergone radical prostatectomy (RP) with peri-prostatic nerve damages, a novel strategy to improve the outcome of such patients was recently proposed performing “nerve transfer”, a surgical technique used in peripheral motor nerve reanimation. In a small group of patients, a neurotization of the dorsal nerve of the penis with femoral nerve was performed through a sural nerve cable graft. The preliminary step of the surgical procedure was tested on rat animal model with a direct motor nerve transfer to corpora cavernosa with encouraging results regarding the feasibility and safety of the surgical technique in which no acute complications were reported.

Nerve decellularization protocol for in vivo implantation
Decellularized nerve graft could represent an alternative strategy to autograft for repairing injured nerves: indeed, decellularized nerve retains the 3D structure useful to sustain axonal outgrowth with the complete removal of immunogenic components. We tested a novel decellularization method to decellularized rat and porcine nerves to study its effectiveness to bridge nerve stumps after peripheral nerve injury. Morphological analysis demonstrated the maintenance of ECM structure and the removal of components that should avoid immunogenic problem in order to study the possible application of acellular pig graft on clinical setting.

Microbiota and peripheral nervous system
We are investigating the correlation between the microbiota and the peripheral nervous system and its target. We analysed peripheral nerves, dorsal root ganglia (DRG) and skeletal muscles of neonatal and young adult mice with the following gut microbiota status: a) germ free (GF), b) gnotobiotic, selectively colonised with 12 known gut bacteria (OMM12), or c) complex gut microbiota (CGM). Morphometrical analysis mainly revealed that absence of gut microbiota impairs the development of median nerves, resulting in smaller diameter and hypermyelinated axons, as well as smaller unmyelinated fibers. Accordingly, DRG and sciatic nerve transcriptomic analysis highlighted a panel of differentially expressed developmental and myelination genes. Moreover, the GF status resulted in

histologically atrophic skeletal muscles, impaired formation of neuromuscular junctions, and deregulated expression of related genes.

Role of the vascularization in the nerve regeneration within different conduits
Repair of severe nerve injuries requires an autograft or a conduit to bridge the gap, but conduit effectiveness is comparable to autograft only for short gaps. Understanding nerve regeneration within short gaps should help improve their efficacy for longer gaps. To this aim we analysed nerve regeneration within empty chitosan conduits, showing that endothelial cells formed a dense capillary network used by Schwann cells to colonize the conduit, followed by axon regrowth. Our data suggest that angiogenesis plays a key role in nerve regeneration providing a pathway for the migration of Schwann cells.

e. Advancement in the field (1000 characters)

The main advancements reached with our research activities of the 2022 can be summarized as follow: i) Promoting vascularization might be a good strategy to support nerve regeneration when angiogenesis is impaired, such as for long-gap nerve injuries, or in elderly patients, or when repair is delayed. ii) We demonstrate for the first time a regulatory impact on proper development of the somatic peripheral nervous system and its functional connection to skeletal muscles, thus establishing the existence of a novel 'Gut Microbiota-Peripheral Nervous System-axis'. iii) we standardized a in vivo experimental model that can be used for studying new strategies to improve the regeneration of the peri-prostatic nerve after radical prostatectomy.

f. Publications[75]

1. Alvites RD, Branquinho MV, Sousa AC, Lopes B, Sousa P, Prada J, Pires I, Ronchi G, Raimondo S, Luís AL, Geuna S, Varejão ASP, Maurício AC. 2022. Effects of Olfactory Mucosa Stem/Stromal Cell and Olfactory Ensheating Cells Secretome on Peripheral Nerve Regeneration. Biomolecules. 2022; 12(6):818.
2. Carta G, Fornasari BE, Fregnan F, Ronchi G, De Zanet S, Muratori L, Nato G, Fogli M, Gambarotta G, Geuna S, Raimondo S. 2022. Neurodynamic Treatment Promotes Mechanical Pain Modulation in Sensory Neurons and Nerve Regeneration in Rats. Biomedicines. 2022; 10(6):1296.
3. Colonna MR, Piagkou M, Monticelli A, Tiengo C, Bassetto F, Sonda R, Battiston B, Titolo P, Tos P, Fazio A, Costa AL, Galeano M, Porzionato A, De Caro R, Cucinotta F, Anastasopoulos N, Papadopulos NA, Geuna S, Natsis K. 2022. Lumbrical Muscles Neural Branching Patterns: A Cadaveric Study With Potential Clinical Implications. Hand (N Y). 2022; 17(5):839-847.
4. Fornasari BE, Zen F, Nato G, Fogli M, Luzzati F, Ronchi G, Raimondo S, Gambarotta G. Blood Vessels: The Pathway Used by Schwann Cells to Colonize Nerve Conduits. Int J Mol Sci. 2022; 23(4):2254.
5. Herrera-Rincon C, Murciano-Brea J, Geuna S. 2022. Can we promote neural regeneration through microbiota-targeted strategies? Introducing the new concept of neurobiotics. Neural Regen Res. 2022; 17(9):1965-1966.
6. Muratori L, Fregnan F, Maurina M, Haastert-Talini K, Ronchi G. 2022. The Potential Benefits of Dietary Polyphenols for Peripheral Nerve Regeneration. Int J Mol Sci. 2022; 23(9):5177.
7. Sureram S, Arduino I, Ueoka R, Rittà M, Francese R, Srivibool R, Darshana D, Piel J, Ruchirawat S, Muratori L, Lembo D, Kittakoop P, Donalisio M. 2022. The Peptide A-3302-B Isolated from a Marine Bacterium Micromonospora sp. Inhibits HSV-2 Infection by Preventing the Viral Egress from Host Cells. Int J Mol Sci. 2022;23(2):947.
8. Kayhan Kustepe E, Altunkaynak BZ, Alkan I, Kivrak EG, Yildiran A, Geuna S. 2022. Potential Effects of Stem Cells Derived from the Peripheral Nerve and Adipose Tissue after the Nerve Crush Injury in Control and Obese Rats. J Invest Surg. 2022; 35(5):1021-1033.

[75] DO NOT include papers in press or submitted.

5. 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 somatic and autonomic peripheral nerve lesions.
The nerve regeneration within different artificial or biological conduits used to repair a nerve gap will be further studied. In particular, chitosan conduits enriched with muscle fibres, silk conduits, "muscle-in-vein" (a well-established technique to repair nerve gaps), will be analysed to assess the role of the different cell populations and factors in the regeneration process, focusing the attention on the role of vascularization, with the aim of improving the outcome when larger nerve gaps are repaired.
To study the peripheral nerve in vitro, a "nerve-on-a-chip" system will be developed, using human induced pluripotent stem cells (hiPSC) cultured on a 3D scaffold developed by bioengineers at the Politecnico of Torino.
In addition, protocols for nerve decellularization will be standardized and in vivo test will be performed to study the ability of the decellularized nerve to sustain nerve regeneration.
To improve the study on the chitosan membrane (FDA approved) applied after cavernous nerve resection, further in vivo study on rat animal model and functional test will be performed to straighten the collaboration with Professor Porpiglia, the head of the Department of Urology in San Luigi Gonzaga Hospital. Finally, the group is going to deepen the innovative research topic focused on the microbiota alteration and its involvement in several peripheral nerve disorders.

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

Improvement of axonal regeneration

Although the PNS has an intrinsic capability to regenerate after trauma, functional recovery is often incomplete and unsatisfactory. A need therefore exists for devising new strategies for promoting the outcome after nerve trauma, especially in cases of severe nerve lesions, when nerve tubulization is needed to bridge proximal and distal nerve stumps. The role played by newly formed blood vessels as a substrate for guiding Schwann cell migration and cord formation within an empty conduit was recently shown, but needs further investigation to understand the role played by the different cell populations (fibroblasts, macrophages, endothelial cells, Schwann cells) not only within empty conduits, but also within conduits enriched with muscle fibres or within "muscle-in-vein".

Development of human peripheral nerve models in vitro

Preclinical models, including both animal and in vitro models, have failed to translate to human, as a significant proportion of clinical trials fail. Reasons for lesser predictivity of animal models are attributed to differences in the underlying biology of the disease in animal versus humans. The development of in vitro microphysiological systems, including organs-on-chips, for mimicking human tissue physiology, are expected to bridge the gap between animal experimentation and predicting the efficacy of the drugs in humans. Animal testing remains the gold standard model also because peripheral nerves lack appropriate human-relevant in vitro models.

Strategies to improve the functional recovery after radical prostatectomy

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

Microbiota and peripheral nervous system

Recent advances in research have described the importance of gut microbiota in influencing not only the gastrointestinal tract, but also a growing list of other organs, highlighting the implication of gut dysbiosis in the development of a number of diseases. Moreover, the relationship between the microbiota and the regeneration process has become a hot topic, in determining microbial taxa

modulating the host tissue regeneration. To date, we demonstrated a direct link between microbiota and somatic peripheral nervous system development, while its impact on regeneration after nerve traumatic injury was only recently reported, albeit with no molecular insight.

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)

- **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, in vivo studies will be performed to evaluate the performance of decellularized nerve graft to improve nerve regeneration and to achieve functional recovery.

- **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 peri-prostatic 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.

- **Studying the relationship between microbiota alterations and peripheral nerve structure, function and regeneration**. We have just demonstrated a regulatory impact of the gut microbiota on proper development of the somatic peripheral nervous system and its functional connection to skeletal muscles. The next step will be to investigate the effect of gut microbiota dysbiosis on Wallerian degeneration and denervation-induced skeletal muscle atrophy as well as on peripheral nerve regeneration and muscular reinnervation in adult mice. Moreover, in vitro analysis will be performed to elucidate the effect of microbiota-derived metabolites on the activity of cells belonging to the neuromuscular system.

- **Development of an in vitro model of bioengineered functional peripheral nerve.** This project aims at developing a "nerve-on-a-chip" system for peripheral nerve studies using human cells. A bioengineered functional nerve is a useful tool for preclinical in vitro assays, because it meets the ethical principles of the 3Rs (Refinement, Reduction and Replacement of animals in research) and, using human cells instead of rodent cells, bypasses the problem of the differences between species. Specific aims of this project will be: 1-to set up in vitro cell cultures of Schwann cells, motor neurons and/or sensitive neurons derived from human induced pluripotent stem cells (hiPSC); 2- to develop a bioengineered "nerve-on-a-chip" by co-culturing human Schwann cells and neurons on a 3D scaffold developed in collaboration with bioengineers of the Politecnico di Torino, to monitor myelination; 3-

to study the effects of different treatments (with drugs, gut bacteria metabolites, …) on cell cultures and on the bioengineered peripheral nerve.

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

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2022

Laboratory name: Ageing and Alzheimer's disease

4. LABORATORY DESCRIPTION – PERSONNEL:[76]

Principal Investigator
Surname, name, position, degree, birthdate, phone, email

TAMAGNO ELENA,
Degree: PhD Birthdate: 14 July 1967

National: Italian Gender: Female

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e-mail: elena.tamagno@unito.it

Position: Full Professor

Role & expertise: Pathogenesis of Alzheimer's disease

Personnel
Surname, name, position, degree, birthdate, phone, email, role & expertise

MICHELA GUGLIELMOTTO

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Role & expertise: Pathogenesis of Alzheimer's disease

VALERIA VASCIAVEO

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Position: PhD student

Role & expertise: Pathogenesis of Alzheimer's disease

GIULIA MORELLO

Degree: PhD student Birthdate: 05/12/1998

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

Email: g.morello@unito.it

Position: PhD student

Role & expertise: Pathogenesis of Alzheimer's disease

Thesis Students

SARA RINALDI

Degree; student

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ALMA FERRO

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VALERIA ROMINA CUSTODIO

Degree; student

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Gender: Female

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Position: Thesis student

LORENZO MINOTTA

Degree; student

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Gender: Male

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Position: Thesis student

GIANLUCA SCATURRO

Degree; student Birthdate: 26/08/1997

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Position: Thesis student

KHOSRO AMINZADEH

Degree; student Birthdate: 12/08/1992

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Position: Thesis student

5. CURRENT GRANTS

Starting-end date	Project Title and ID	Beneficiary[77]	Funding Program/Agency	Role of the unit[78]	Overall Amount Funded	Managed by FCO/UNITO

6. SCIENTIFIC ACTIVITIES IN 2022

Name, Role (PI)

Supervised PhD students:	
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	
• Invited talks:[79]	
• Science communication:[80]	
• Editorial duties:	

[77] 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.

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

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

[80] Public engagement

• others[81]	
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Other organizational activities:[82]	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Name, Role[83] xxx

Supervised PhD students:	
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	
• Invited talks:	
• Science communication:	
• Editorial duties:	
• others	
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

ALL LAB MEMBERS

Activities:[84]	

[81] Posters at meetings, participation in the board of scientific societies, referee for grant agencies
[82] No university appointments.
[83] Please duplicate the module for the various lab members
[84] 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 2022[85]

a. Summary (500 characters)

Our group is involved in studying the cellular and molecular mechanisms associated with aging that cause Alzheimer's disease, in order to contribute to the development of new therapies. In this context, we focused our attention on the study of different biomarkers and risk factors of the disease. Thus, Alzheimer's disease pathology begins many years before its symptoms; therefore, we must take advantage of this long window to modify and modulate all known risk factors for the disease.

b. Background and rationale (3000 characters)

Alzheimer's disease (AD) is considered the leading cause of dementia and is becoming one of the most expensive and deadly diseases of our time. Thus, it is estimated that 50 million people worldwide endure dementia, and this number is set to rise to 152 million in 2050. Moreover, Alzheimer's patients need specialized and expensive care, the annual cost of treatment worldwide is around a trillion US dollars, and it is predicted that this cost will significantly increase by 2030. In Italy there are an estimated 600,000 cases.
The pathophysiology of the disease is complex and multifactorial and certainly not entirely known. There are two markers of the disease. One is β amyloid (Aβ), which accumulates abnormally in AD brain tissues and forms extracellular plaques known to induce synaptic alterations and neurodegeneration. The other is Tau protein, which forms intracellular neurofibrillary tangles that are also responsible for neurodegeneration.
The only 4 available Food and Drug Administration (FDA) approved agents for AD treatment offered limited effects on cognitive improvement. Though considerable efforts have been directed to tackle this disease, AD remains inexorable and incurable. The high failure rate of AD drug development was thought to be mainly due to our poor knowledge about the complex pathological mechanism of this disease. There are numerous factors playing a role in the prognosis of AD. A number of hypotheses concerning the root cause of AD reveal the complexity of the disease. Cholinergic deficiency, amyloid beta (Aβ) toxicity, tau protein hyperphosphorylation, synaptic dysfunction, oxidative-stress, and neuroinflammation were proposed to be responsible for AD development. Regardless what the root cause of AD is, all these factors intensify the progression of disease. For decades, the "one drug for one target" strategy has been dominant, but is still unable to conquer this multifactorial disease. It is hypothesized that the multifunctional strategy, which could simultaneously modify different pathological pathways, would be helpful to treat this multifaceted disease.
Although a number of promising therapeutic strategies have been evaluated, more extensive and intensive fundamental studies are still needed. Considering the complexity of AD pathology, multifunctional agents designed with multitarget potential could lead to a breakthrough in AD therapeutic development. Preclinical studies on different pathologies and multitarget treatments may provide a pool of lead compounds for future clinical investigations. There is no royal road to overcome AD, but multifunctional drug is likely to give hope for AD treatment.

c. Objectives (1000 characters)

In our laboratory we try to deepen the knowledge on the pathogenesis of Alzheimer's disease, in particular regarding the relationship between beta amyloid and tau protein. We are still trying to understand how some known risk factors for the disease act at the molecular level, in particular sleep disorders can act at the molecular level. Thus, it was recently reported that sleep is an important physiological process, during which extracellular metabolic wastes, such as amyloid and tau protein, are cleared via paravascular pathway. The brain relies on the glymphatic clearance pathway to remove these waste materials. The impairment of glymphatic pathway function in the aging brain slows the clearance of interstitial Aβ, rendering the aging brain vulnerable to neurodegenerative disease.

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

Changes in the timing and structure of sleep occur across the lifespan. Increased sleep fragmentation and reductions in slow wave sleep (SWS) represent the hallmark signs of age-related changes in sleep.

d. Results (4000 characters)

We obtained some interesting results.

- Validation of sleep fragmentation protocol through electroencephalography (EEG) recordings in 5XFAD and control mice models
  The aim of our sleep fragmentation protocol was to achieve a chronic state of sleep fragmentation, without significantly impairing the total amount of sleep. The hypnograms obtained in normal conditions and during sleep fragmentation periods were analyzed and as expected, both the wild type (wt) and the 5xFAD strains showed a significant increase of sleep/wake shifts.
- Sleep fragmented 5xFAD mice show an accentuate anxious behavior analyzed by the Elevated Plus Maze (EPM) and the Open Field Test (OFT).
  Sleep fragmentation increased anxiety in both strains of mice by increasing hyperactivity as shown in the total distance travelled in arena, which is greater in the closed arms than in the open arms.
- Sleep fragmentation accelerates AD progression by enhancing Aβ accumulation and inducing tau phosphorylation in 5xFAD mice.
  As for 5xFAD mice, this strain at 2 months of age already displays visible extracellular Aβ accumulation, thus we explored whether this accumulation could be more emphasized after the disruption of sleep. We showed that in fragmented 5xFAD mice compared to control, Aβ accumulation increased in both the cortexes and the dentate gyrus, as well as in all the other regions also involved in sleep regulation. Interestingly, Aβ accumulation increased also in the lateral septum, a brain region which modulates cognitive processing in the cortex and hippocampus. We also observed an initiation of tau phosphorylation in the dentate gyrus in 5xFAD mice after sleep fragmentation compared to the not fragmented mice, where tau phosphorylation is not observed.
- Sleep fragmentation induces neuroinflammation by activating microglia and consequently astrocytes.
  Neuroinflammation is known to occur in AD pathology. To validate an activation of the neuroinflammation mediated by sleep disruption, we analyzed by immunofluorescence the density of astrocyte cells. Indeed, GFAP+ signal increased in all the areas analyzed in F-5xFAD mice compared to control, thus indicating a possible astrogliosis. Interestingly, this signal well correlates with the increase of Aβ plaque accumulation.mTo confirm this result, we also investigated the activation of microglia, which is known to activate astrocytes by the release of immune factors. Here, we observed a major activation of microglia in F-5xFAD mice compared to control in all the brain areas analyzed. This activation is notable by analyzing the morphological complexity of microglia cells. By using Fiji software, we firstly skeletonized every cell taken in exam, and analyzed them by AnalyzeSkeleton(2D/3D) ImageJ plugin. In sleep fragmented mice, iba1+ cells are more activated by comparing the number of cell branches, branch junctions, and the voxel end-points which significantly decreased in most of the regions analyzed compared to control, in which microglia cells are less activated and consequently more ramified.
- Sleep fragmentation differently influences AQP4 expression according to the severity of the disease.
  One of the clearance pathways of Aβ plaques is displayed by the glymphatic system, in particular by the activity of the AQP4 channel, located in the end-feet of astrocytes surrounding vessels. Since we observed an augmentation of Aβ accumulation mediated by sleep disruption, we investigated whether this clearance system is compromised. In 2-month-

old 5xFAD mice, we observed an increase in the density of AQP4+ signal in all the brain areas involved. But despite the augmentation of Aβ plaques, we detected the AQP4 signal in the perivascular areas in both the control and the fragmented mice, thus indicating a possible functional channel activity. Interestingly, in older mice (6-month-old) we observed a decrease of AQP4+ signal, which could be due to a decrease in astrocytes.

e. Advancement in the field (1000 characters)

We performed and validated a mouse model of AD and sleep fragmentation, which properly mimics a real condition of intermittent awakening. We noticed that sleep fragmentation induces a general acceleration of AD progression in 5xFAD mice, while in wild type mice it affects cognitive behaviors in particular learning and memory. Both these events may be correlated to aquaporin-4 (AQP4) modulation, a crucial player of the glymphatic system activity. Nevertheless, an in-depth study is needed to better understand the mechanism by which AQP4 is modulated and whether it could be considered a risk factor for the disease development in wild type mice. If our hypotheses are going to be confirmed, AQP4 modulation may represent the convergence point between AD and sleep disorder pathogenic mechanisms. there is an urgent need to identify early biomarkers that determine which individuals are at greatest risk for AD development, motivated by at least two goals: (1) offering the chance for preventive measures, in the pre-disease onset phase, and (2) allowing nascent treatment intervention, early in the disease process.

f. Publications[86]

1) Vasciaveo V, Iadarola A, Casile A, Dante D, Morello G, Minotta L, Tamagno E, Cicolin A, Guglielmotto M. 2023 Sleep fragmentation affects glymphatic system through the different expression of AQP4 in wild type and 5xFAD mouse models. Acta Neuropathol Commun. Jan 18;11:16.

2) Tamagno E, Guglielmotto M. 2022 Estrogens still represent an attractive therapeutic approach for Alzheimer's disease. Neural Regen Res. Jan;17:93-94.

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):

Alzheimer's disease (AD) is a devastating neurodegenerative disorder that results in loss of memory and cognitive function, eventually leading to severe dementia. Fasting can be relevant since, recently, studies have shed light on its role in adaptive cellular responses that reduce oxidative damage and inflammation, optimise energy metabolism, and strengthen cellular protection. Fast-mimicking diet (FMD), and re-feeding periods, promote regenerative processes and amelioration of dysfunctional neurons, leading to improvement of symptoms in mice and humans. The wide-range effects of FMDs on metabolic, inflammatory and regenerative pathways have the potential to ameliorate the pathology and symptoms of Alzheimer's disease (AD). The aim of the project is to investigate the crosstalk between Aβ and Tau that represent a crucial node in the study of the pathogenesis of Alzheimer's disease related to FMD. Likewise, to explore a new possibility to prevent neurodegeneration in dementia, studying the fast-induced molecular mechanisms that can highlight new

[86] DO NOT include papers in press or submitted.

pathways and macromolecules involved in the prevention of AD and promote longevity benefit effect. On these bases, we have developed, and behaviorally and histopathological characterised a 5xTg-AD/hTauTg mouse model that mimics the pathological evolution of AD ("-amyloid production /wild type htau expression) to investigate the effect of FMD cycles. We aim to demonstrate that nutrition represents a modifiable environmental factor that could strongly impact AD pathology by modulating its phenotypic expression.

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

Based on the existing evidence from animal and human studies described, we know today that there is great potential for lifestyles that incorporate intermittent fasting (IF) or periodic fasting (IP) during adult life to promote optimal health and reduce the risk of many chronic diseases, particularly for those who are overweight and sedentary. In this regard Fasting regimens can ameliorate disease processes and improve functional outcome in animal models of disorders that include cancer, myocardial infarction, diabetes, stroke, AD, and PD.
When we refer to those metabolic-approaches we have to describe: Fasting is distinct from caloric restriction (CR), in which the daily caloric intake is reduced chronically by 20%–40%, but meal frequency is maintained. Starvation is instead a chronic nutritional insufficiency that is commonly used as a substitute for the word fasting, particularly in lower eukaryotes, but is also used to define extreme forms of fasting, which can result in degeneration and death. Caloric restriction more generally on senescent cells display beneficial effects as an intervention method for reducing the age-associated chronic disease such as AD, and enhancing lifespan.
Also, we can say that to a large extent CR can prevent senescence and this can happen on senescent cells because of the up-regulation of the defence program partly due to the sirtuins function and in part through up-regulation of FOXO gene. whereas we can report other mechanisms involved in prevention of senescence, acting because CR, such as the enchantment of non-homologous end joining (NHEJ), DNA repair system that can aid in solving DNA lesions, Supporting evidence show that CR can modulate positively the initiation and progression of amyloidogenesis through potential catalytic role of ADAM10, part of ADAM proteinase family, involved in α-secretase activity. CR dietary regimen can influence α-secretase production as a consequence of sAPPα neurotrophic action, enhancing the brain repair action and its neuroprotective role. Highlighting the fact that dietary regimen has a role as future preventative measure aimed at delaying the onset of AD, and amyloid deposition.
So, taken together all the previous consideration, there is a significant amount of data suggesting that pursuing the way of novel treatment, based on control of diet, specifically reducing the calorie intake, given by intermittent fasting recognized as a modifiable factor, could have a beneficial impact slowing down the pathogenic onset of AD.

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

Alzheimer, Huntington, multiple sclerosis, and amyotrophic lateral sclerosis: these are some of the most commonly known neurodegenerative diseases. The road towards their cure inevitably starts from basic research capable of understanding the molecular and cellular mechanisms which underlie their pathogenesis. For this reason, research at NICO is devoted to investigate the normal structure and function of the central nervous system, along with neurodegenerative events and reparative/regenerative processes of nerve and glial cells. Our basic studies on the pathogenesis of Alzheimer's disease are perfectly in line with the mission of the institute

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

The aim of this project is to investigate the molecular effect of the Fast-Mimicking diet on 5xTg-AD/hTauTg mouse model. As AD is a multifactorial disease we cannot reduce or diminish the effect of genetic factors or medical factors such as the onset of cancer, or ageing consequences, neither gender, we can take actions on reducing negative influence of environmental or lifestyle factors.

The main goal is to confront the effect both at molecular level and behavioral one, trying to find a prevention strategy for reducing the hallmarks of AD and senescent cell, in which accumulation of amyloid beta plaque and neurofibrillary tangles are predominant causing neuronal loss and cognitive decline.
The fasting mechanisms would eventually reduce the accumulation of misfolded proteins and increase the healthy status of the cells for a longer period of time, restoring a healthier condition also in aged people, or delaying the time by which the AD will onset. The major proteins involved in the pathological role of AD can be ascribed as Amyloid- beta and Tau proteins. In order to prove changes in the level of those pathological forms of proteins we have performed several experiments, trying to quantify their levels and how much they are involved in the pathogenesis.

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

Alzheimer's disease is the most common neurodegenerative disease. Due to the increase in life expectancy, AD, which affects the older population, has become a pressing problem. Almost 1 million Italians suffer from this terrible disease. With no new discoveries, this balance sheet reflects only the tip of the iceberg. In fact, cases are expected to triple in the next generations. Currently, AD is an invariably fatal disease, without treatment, the direct and indirect costs of which will become unsustainable for the health system. Studying the weight of risk factors, such as the loss of sleep quality, which are largely reversible, will help to design new, increasingly personalized therapies, capable of stopping the disease or at least drastically alleviating its symptoms.
Since the incidence of Alzheimer's disease increases exponentially with age, a dramatic increase in its prevalence (number of cases / inhabitants) is expected with an enormous socio-economic cost, soon unsustainable. In the European Union, the World Health Organization has predicted that 88 million people will die from a chronic disease. The percentage of the population over the age of 65 (general retirement age) will increase from 90 million in 2012 to 155 million in 2060. Among people over 65, the over 80s segment is the one that has experienced the greatest growth (from 0 , 6% in 1901 to 3.9% in the 2000s and 11.9% is expected in 2050). The large and growing presence of elderly people suffering from Alzheimer's disease, together with the lack of therapies, make this disease one of the diseases with the most serious social impact in the world. It is increasingly necessary to understand the early mechanisms underlying the disease, in order to prevent, or at least delay, its onset.

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

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2022

Laboratory name: Neurophysiology of neurodegenerative diseases

1. LABORATORY DESCRIPTION – PERSONNEL:[87]

Principal Investigator
Surname, name, position, degree, birthdate, phone, email
TEMPIA Filippo, Full Professor, MD PhD, 20/08/1960, +39-011-670-6609, filippo.tempia@unito.it

Personnel
Surname, name, position, degree, birthdate, phone, email, role & expertise
HOXHA Eriola, Associate Professor, PhD, 26/01/1981, +39-011-670-6609, eriola.hoxha@unito.it, supervision, patch-clamp, molecular biology

MONTAROLO Francesca, postdoc fellow, PhD, 14/05/1983, +39-011-670-6609, francesca.montarolo@unito.it, behavioral experiments, histology, molecular biology, patch-clamp

ROMINTO Anita Maria, PhD student, MS, 19/07/1996, +39-011-670-6609, anitamaria.rominto@unito.it, behavioral experiments, histology, molecular biology, patch-clamp

2. CURRENT GRANTS

Starting-end date	Project Title and ID	Beneficiary[88]	Funding Program/Agency	Role of the unit[89]	Overall Amount Funded	Managed by FCO/UNITO
01/07/22-31/08/23	Ataxia Telangiectasia: a new murine model to discover the connection between Purkinje cell calcium homeostasis disruption and disease pathogenesis	Eriola Hoxha	ANAT	PI	€ 15.000	UNITO

3. SCIENTIFIC ACTIVITIES IN 2022

Tempia Filippo, Role (PI)

Supervised PhD students:	1: Ilaria Balbo
• International collaborations:	Prof. Fernanda Laezza, University of Texas Medical Branch, USA
• 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

[87] For further personnel copy the corresponding form, and number accordingly; do not exceed one line to describe role & expertise
[88] 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.
[89] Coordinator/PI of research unit/team component.

Organizational activities and responsibilities at NICO:	Group Leader of Neurophysiology of Neurodegenerative Diseases; Director of the NICO Animal Facility
Technology transfer achievements (patents, etc.):	1 patent application (with Eriola Hoxha)

Eriola Hoxha, Supervisor and Researcher [90]

Supervised PhD students:	1: Ilaria Balbo
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	-Prof. Shan Zha, (Dept. of Pathology and Cell Biology), Columbia University, New York, USA. -Prof. Jeanne Nerbonne, (Dept. of Developmental Biology) Washington University School of Medicine, St. Louis, MO, USA -Prof. Dorota Skowronska-Krawczyk, PhD (Dept of Physiology and Biophysics Dept of Ophthalmology, Center for Translational Vision Research School of Medicine UC Irvine
• Invited talks:	• *Ataxia Telangiectasia: a new murine model to discover the connection between Purkinje cell calcium homeostasis disruption and disease pathogenesis.* September 24th, 2022, in the frame of the meeting: "A-T Family Weekend 2022", September 23-25, 2022, Lainate, Italy. Invited oral communication. • *Role of membrane lipid composition in synaptic transmission.* September 16th, 2022, in the frame of the 72nd National Congress of the Italian Society of Physiology. September 14-16, 2022, Bari, Italy. Oral communication.
• Science communication:	
• Editorial duties:	Editor of Frontiers in Aging Neuroscience; Member of the editorial board of Frontiers in Cellular and Molecular Mechanisms of Brain-aging; Guest Associate Editor of Frontiers in Cellular Neuropathology, for the Topic "The cerebellar involvement in non cerebellar pathologies" in Frontiers in Cellular Neuropathology.
• others	
Organizational activities and responsibilities at NICO:	Responsible for the water ultrapurification systems at NICO
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	1 patent application (with Filippo Tempia)

[90] Please duplicate the module for the various lab members

4. Research activity in 2022[91]

a. Summary (500 characters)

Based on the results of the study about GSK3 in blood samples from patients with depression, one patent application has been filed with a method for the differential diagnosis between major depression and bipolar disorder. In a novel murine model of ataxia-teleangiectasia we found early mortality and motor deficits consistent with the disease. Advances have been obtained also on Kv7 channels.

b. Background and rationale (3000 characters)

1. The molecular mechanisms of depression are not clearly 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. Furthermore, a biological marker to distinguish Major Depressive Disorder (MDD) from Bipolar Disorder (BD) is needed but still unavailable.

2. Ataxia-teleangiectasia (AT) 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 Purkinje cells (PCs) with a compromised cerebellar structure. The 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 AT cerebellum in early asymptomatic phase showed a possible compromised cerebellar glutamatergic signaling paralleled by a deranged calcium homeostasis, which might be involved in the mechanism of PCs death and ataxia.

3. Regulation of the resting membrane potential and the repolarization of neurons are important in regulating neuronal excitability. The M-current (I_M) is a slowly deactivating, non-inactivating potassium current due to Kv7 channels encoded by members of the *KCNQ* gene family *KCNQ1–KCNQ5*. Kv7 currents have not yet been studied in the cerebellar Purkinje cell (PC). 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 this cell type and in cerebellar physiology.

c. Objectives (1000 characters)

Aim 1: Role of GSK3 in mood disorders.
Subaim 1.1: GSK3 alterations in patients with mood disorders. The aim was to evaluate the total and phosphorylated protein levels of GSK3α and GSK3β in blood samples from patients with MDD or BD.
Subaim 1.2: Neuronal mechanisms of mood disorder in mice models of depression. The goal was to establish single cell recordings of pyramidal and non-pyramidal neurons of the medial prefrontal cortex of mice models of mood disorders.

Aim 2. The first goal was to detect the age of onset and the severity of motor symptoms in the new murine model of A-T. The second goal was to search for alterations in PC action potential firing in A-T model mice.

Aim 3: to study the role and the physiological functions of Kv7 channels in cerebellar PCs.

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

d. Results (4000 characters)

Aim 1: Role of GSK3 in mood disorders.

Subaim 1.1: Proteins extracted from peripheral blood mononuclear cells of patients with depression were analyzed with the western blot technique using antibodies for total GSK3α and GSK3β, and for their phosphorylated forms. The results were markedly different in patients with unipolar depression (UD) in major depressive disorder (MDD) compared to patients in a depressive episode of bipolar disorder (BD). While in UD patients total GSK3α and GSK3β level were indistinguishable from healthy control subjects, UD patients showed significantly lower levels of GSK3β and a trend to lower GSK3α. The phosphorylated forms of GSK3 were similar in all groups. Based on these results on total GSK3α and GSK3β, we submitted an Italian national patent application to use this data to guide the differential diagnosis between UD and BD (application number 102022000024564 of 29/11/2022).

Subaim 1.2: Neuronal mechanisms of mood disorder in mice models of depression. For this study we focused on the medial prefrontal cortex (mPFC), including anterior cingulate, prelimbic and infralimbic areas. This is the brain region with the greatest changes in activity in patients with depression and the principal target area for therapeutic transcranial or deep brain stimulation to relieve depression. We obtained the first recordings in our laboratory of neurons in this area.

Aim 2. We found that A-T model mice display significant motor deficits already at 2 months of age. At the same age we recorded in PCs the spontaneous action potential firing, which was the same as in control animals. We also started the analysis of the parallel fiber/PC synapse, and the preliminary result suggest that it is unaltered.

Aim3. We showed that PCs express Kv7.2 and Kv7.3 channels in the cell body. Moreover, Kv7 potassium channels strongly modulate PC neuronal excitability. A Kv7 antagonist enhances evoked neuronal firing, while a Kv7 activator decreases evoked action potentials.

e. Advancement in the field (1000 characters)

The finding of lower levels of total GSK3β in peripheral blood mononuclear cells of patients with BD, but not of UD, indicates different mechanisms in these two forms of depression. Furthermore, such a difference can be exploited to correctly diagnose patients with depression so that the correct therapy can be started.

Information about the mechanism of cerebellar degeneration in AT patients is still lacking. We expect that this project will be an important advancement of the knowledge, on the way, eventually leading to the discovery of a really effective treatment for patients with AT.

Kv7 potassium channels had not yet been studied in PCs; we show for the first time their involvement in the control of membrane excitability in this cell type.

f. Publications[92]

1. Balbo I, Montarolo F, Genovese F, Tempia F, Hoxha E. 2022 Effects of the administration of Elovl5-dependent fatty acids on a spino-cerebellar ataxia 38 mouse model. Behav Brain Funct. 18, 8. https://doi.org/10.1186/s12993-022-00194-4
2. Cristiano C, Hoxha E, Lippiello P, Balbo I, Russo R, Tempia F, Miniaci MC. 2022 Maternal treatment with sodium butyrate reduces the development of autism-like traits in mice offspring. Biomedicine &

[92] DO NOT include papers in press or submitted.

Pharmacotherapy. 156: 113870. https://doi.org/10.1016/j.biopha.2022.113870

3. Montarolo F, Martire S, Chiara F, Allegra S, De Francia S, Hoxha E, Tempia F, Capobianco MA, Bertolotto A. 2022 NURR1 deficient mice have age- and sex-specific behavioral phenotypes. J Neurosci Res. 100:1747-1754. https://doi.org/10.1002/jnr.25067

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 plan to extend the study on the role of the GSK3 kinase in patients with mood disorders. In animal models of depression with hyperactive GSK3, by patch-clamp experiments we plan to search for alterations in neuronal excitability and synaptic transmission in the prefrontal cortex, which is a main hub of the network implied in mood disorders. In these models, neuronal activity will be recorded also by fiber photometry in behaving animals. In a novel murine model of ataxia-teleangiectasia we plan to identify early neuronal dysfunctions including calcium signaling and synaptic transmission in the cerebellar cortex. Kv7 potassium currents will be studied in cerebellar Purkinje cells and their functional role will be assessed and used as a basis to understand diseases in which Kv7 channels are involved

b. 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 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. Lower GSK3 level are specific of patients with BD relative to MDD. Mutant mice with a constitutive GSK3 hyperactivity have increased susceptibility to depression, but the molecular and electrophysiological mechanisms are not known. Moreover, the role of GSK3 in the brain regions involved in depression is still unknown.
mood disorders but its activity and the specific role in MDD relative to BD are still unknown..
2. 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. The Atm protein is involved in several cellular responses to damage, including ionizing radiations, oxidative stress, hypoxic ischemia. In spite of such knowledge on the role of Atm in response to cell stress, the mechanisms that cause PC death are still unknown. Recent indirect evidence suggests a role of excitotoxicity and dysregulation of calcium signaling. Our aim is to exploit a novel animal model of A-T to uncover the mechanisms causing PC lesion. The research will focus on the early alterations in PC physiology, including postsynaptic events and calcium signaling.

3. Regulation of the resting membrane potential and the repolarization of neurons are important in regulating neuronal excitability. The M-current (I_M) is a slowly deactivating, non-inactivating potassium current due to Kv7 channels encoded by members of the *KCNQ* gene family *KCNQ1–KCNQ5*. Mutations in *KCNQ2* or *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. Therefore, 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. 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 this cell type and in cerebellar physiology.

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

Our projects regard the neuronal bases of several psychiatric and neurologic disorders. A general aim about psychiatric disorders is to identify new signaling pathways, including the GSK3 kinase, involved in mood disorders. Regarding neurologic diseases, we plan to focus on the neurophysiological basis of A-T. In the realm of physiological mechanisms, 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. The mission of the Institute is exactly the same as ours, namely to advance scientific knowledge regarding brain disorders, including psychiatric diseases such as depression and neurologic diseases like cerebellar ataxias, and physiological functions implied in brain disorders.

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

Aim 1: Neuronal mechanisms involved in mood control by Gsk3 in murine models of depression. The aim of the project is to identify changes in neuronal function induced by Gsk3 dysregulated activity in the mPFC of murine models of depression. For the induction of a depressed-like behavior in mice (henceforth called "depression", referring to individuals who develop a depressed-like behavior) we will use the chronic social defeat stress (CSDS) paradigm. Our previous results showed that Gsk3β becomes hyperactive in mice susceptible to CSDS. Thus, after induction of a depressed-like behavior by CSDS, a thorough evaluation of neuronal activity in the mPFC will be performed.
In addition to the experiments in normal mice after CSDS, in order to identify which changes in neuronal function are caused by Gsk3 hyperactivity we will study mPFC neuronal activity in knock-in mice (abbreviated Gsk3-KI) in which regulatory serines of Gsk3α and Gsk3β have been mutated into alanines, so that both isoforms cannot be inhibited and are constitutively hyperactive (McManus et al., 2005). Gsk3-KI mice under basal conditions (not subjected to a stress protocol) do not display spontaneous anxiety or depressed-like behavior. However, they are highly susceptible to depression in response to stress protocols. An electrophysiological analysis will be conducted in CSDS and Gsk3-KI mice. To study neuronal dysfunction related to mood disorders we'll record action potential firing in slices of mPFC 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 following CSDS.

Aim 2: What are the mechanisms leading to PC death in A-T? The experiments will address the hypothesis of excitotoxicity and of altered calcium signaling. Glutamatergic and GABAergic responses of PCs will be investigated. Calcium signaling will be studied in vivo by two photon microscopy and in vitro by calcium imaging in cerebellar slices. The response of PCs to cellular stress will be studied by application of glutamate agonists and by oxygen/glucose deprivation. Under these conditions the mechanisms of cell degeneration will be investigated.

Aim 3. Identification and physiological roles of Kv7 potassium channels in PCs. Our project about Kv7 channels is aimed at identifying the subunits expressed in PCs and the age at which this expression begins, 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.

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

1. By the experiments of Aim1 we expect to identify GSK3 alterations involved in mood disorders. A possible clinical impact is the possibility to utilize a GSK3 dosage assay to guide and refine the diagnosis.

We expect to characterize the GSK3-modulation profile of different mood disorders and the effects of therapy. The study of action potential firing in the prefrontal cortex would 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. In ataxia-teleangiectasia, excitotoxicity and/or calcium dysregulation might be the mechanism of PC death. The identification of the cell death mechanism would open the way to design new treatments to rescue PCs and prevent ataxia in patients with AT.
3. 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.

f. 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 based 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 available at NICO. We plan to record neuronal activity during induction of depression to detect the neuronal signals involved in mood disorders.
We plan to perform some of the measures of neuronal activity by in vivo two photon imaging. 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.

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2022

Laboratory name: Brain development and disease

1 LABORATORY DESCRIPTION – PERSONNEL:[93]

Principal Investigator
Vercelli, Alessandro, Full Professor, MD PhD, 09/07/1961, +390116706617, alessandro.vercelli@unito.it

Personnel
Boido, Marina, Associate Professor, PhD, 06/09/1980, +390116706613, marina.boido@unito.it, Spinal cord injury, motor neuron diseases (ALS and SMA), drug repositioning, stem cells

Ceccarelli, Adriano, Associate Professor, MD PhD, 28/10/1957, +390116705409, adriano.ceccarelli@unito,it, Molecular biology

Calì, Corrado, Assistant Professor RTD-B, PhD, 27/11/1982, +390116703447, corrado.cali@unito.it, Glia, astrocytes, 3D electron microscopy, 3D modeling and analysis, VR (Virtual Reality), AR (Augmented Reality)

Marvaldi, Letizia, Assistant Professor RTD-B, PhD, 23/01/1983, +390116706632, letizia.marvaldi@unito.it, pain and neurite outgrowth signalling, neurogenetics of pain and neuronal regeneration and survival, importins.

Schellino, Roberta, Assistant Professor, RTD-B (since November 2022), PhD, 11/02/1985, +390116706632, roberta.schellino@unito.it, Neurogenesis, spinal muscular atrophy, Huntington's disease, neuromuscular disease, histology, confocal imaging, behavior

Stanga, Serena, Assistant Professor RTD-B, PhD, 03/06/1983, +390116706632, serena.stanga@unito.it, Ageing and Alzheimer's disease, Motor Neuron Diseases, molecular neuroscience, primary neuronal cultures, mitochondrial dysfunctions

Menduti, Giovanna, Post-doc fellow, PhD, 14/04/1991, +390116706632, giovanna.menduti@unito.it, Cellular and molecular neurobiology, spinal muscular atrophy, drug repositioning

Mezzanotte Mariarosa, Post-doc fellow, PhD, 19/08/1985, +390116706632, mariarosa.mezzanotte@unito.it, Ageing and Alzheimer's disease, brain iron metabolism, histological and molecular analysis

Caretto, Anna, PhD Student, Master degree in CTF, 08/07/1995, +390116706632, anna.caretto@unito.it, Spinal muscular atrophy, neuromuscular diseases

Dallere, Sveva, PhD student, Master degree in Medical Biotechnology, 23/07/1997, +390116706632, sveva.dallere@edu.unito.it, Cell culture, muscular atrophy, Alzheimer disease, iPSCs, terpenes

Pavarino, Gianna, PhD Student, Master degree in Molecular Biotechnology, 02/05/1997, +390116706632, gianna.pavarino@unito.it, Spinal muscular atrophy, depression, molecular biology, iPSCs, terpenes

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

Rasà, Daniela Maria, PhD student, Master degree in Biology, 11/09/1990, +390116706632, danielamaria.rasa@unito.it, Cell culture, motor neuron diseases (SMA and ALS), stress, drug repositioning

Ramanbhai Parmar, Amishaben, PhD Student, Master degree in Pharmacology and Toxicology, 20/12/1988, +390116706632, amishabenramanbhai.parmar@unito.it, Neuronal regeneration and survival

Chiappini, Vanessa, Fellowship recipient, Master's Degree in Biomedical Engineering, 23/07/1997, +390116706632, vanessa.chiappini@unito.it, Spinal cord injury, bioprinting, cell culture

De la Morena Saavedra, Silvia, Fellowship recipient, Master degree in Biomolecules and cell dynamics, 10/06/1998, +390116706632, s.delamorenasaavedra@unito.it, ALS, cell therapy

Ruatti, Cristina, Fellowship recipient, Master's Degree in Medical Biotechnology, 30/04/1997, +390116706632, cristina.ruatti@edu.unito.it, Spinal muscular atrophy, molecular biology

2 CURRENT GRANTS

Starting-end date	Project Title and ID	Beneficiary[94]	Funding Program/Agency	Role of the unit[95]	Overall Amount Funded	Managed by FCO/UNITO
2022	SMA	Vercelli A.	SMArathon	Coordinator	14,000 €	FCO
2022	SMA	Vercelli A.	Girotondo Onlus	Coordinator	20,000 €	FCO
2021-23	Evaluation of new compounds to sustain muscular innervation and trophism	Vercelli A./Boido M.	Pharmafox – M. Hildigher	Coordinators	80,000 €	FCO
2022-2023	Lab-on-chip per una medicina predittiva e di precisione nel neurocovid	Vercelli A.	Regione Piemonte (INFRA-P realizzazione, rafforzamento e ampliamento Infrastrutture di ricerca pubbliche)	Coordinator	160,000 €	UNITO

[94] 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.
[95] Coordinator/PI of research unit/team component.

2021-2022	The involvement of the small heat shock protein HSPB8 in amyotrophic lateral sclerosis	Vercelli A.	AFM Telethon	Coordinator	36,000 €	UNITO
2022-2026	D34H	Vercelli A.	PNRR MUR/MH	Coordinator UNITO	4,3 M€	UNITO[96]
2023-2027	Department of Excellence	Vercelli A.	MUR	Coordinator	7.75 M€	UNITO[97]
2022-2023		Vercelli A.	Theoresi	Coordinator	100,000 €	FCO[98]
2019-2022	The role of SMN protein in translation: implications for Spinal Muscular Atrophy; ID GGP19115 A	Boido M.	Fondazione Telethon	P.I. of research unit	83.600 €	FCO
2021-2023	La bio-stampa 3D: neurobiologia e ingegneria unite per studiare e curare le lesioni al midollo spinale. ID 2020.1801	Boido M.	Fondazione CRT	Coordinator	30,000 €	UNITO
2022-2023	Lab-on-chip per una medicina	Boido M.	Regione Piemonte (INFRA-P realizzazione,	P.I. of research unit	50,000 €	FCO

[96] To this project participate also A. Buffo and F. Tempia

[97] The funding for the department of excellence to the Department of Neuroscience is coordinated by AV, but involved all members of the DNS, and will serve to potentiate infrastructures and personnel

[98] This funding has been obtained together with prof. D. Garbossa, chief of Neurosurgery Clinic

	predittiva e di precisione nel neurocovid		rafforzamento e ampliamento Infrastrutture di ricerca pubbliche)			
2023-2025	A combinatorial pharmacotherapeutic approach to counteract Spinal Muscular Atrophy	Boido M.	AFM Telethon	P.I. of research unit	90,000 €	UNITO
2023-2025	Uncovering the mechanism of action and synergistic potential of a SMA repositioned therapy	Boido M. (grant coordinator, 150 k€ with the other partners)	SMA Europe	Coordinator	60,000 €	FCO
2023	New strategy to prevent the progression of neurodegeneration occurring in Amyotrophic Lateral Sclerosis	Boido M.	Grant for internationalization (GFI) UniTA	Coordinator	12,500 €	UNITO
2022-2025	NODES - INNDIANA	Boido M.	PNRR	P.I. of the research unit	TBD	UNITO
2021-2024	Validazione preclinica di protocolli di inoculo di cellule staminali neurali umane	Boido M	Fondazione Revert	Coordinator	41,000 €	FCO

	(hNSCs) per lo sviluppo di terapie cellulari per pazienti affetti da sclerosi laterale amiotrofica					
2023	Correlative Analysis between Astrocytes and Dendritic Bundles in a Human Cortex EM Dataset	Calì C.	Grant for Internationalization GFI - UNITO	Coordinator	15,000 €	UNITO
2022-2024	Project Title: QEMSK	Calì C.	IonQ Research Credit Program	Coordinator	10,000 €	UNITO
2022-2025	NODES - TINCARE	Calì C.	PNRR	P.I. of the research unit	TBD	UNITO
2022-2024	Braccio di ferro con la demenza: ferro e mitocondri come nuovi target contro la Malattia di Alzheimer	Stanga S.	Fondazione CRT	Coordinator	20,000 €	FCO
2023	Study of human iPSC-derived neural stem cells to unravel molecular mechanisms related to early	Stanga S.	Grant for internationalization (GFI)	Coordinator	15,233 €	UNITO

	mitochondrial dysfunctions and iron dyshomeostasis in neurodegenerative diseases					
2023	New strategy to prevent the progression of neurodegeneration occurring in Alzheimer's disease	Stanga S.	Grant for internationalization (GFI) UniTA	Coordinator	12,500 €	UNITO
2023	Studio delle alterazioni del sistema glicinergico in un modello murino di Atrofia Muscolare Spinale (SMA)	Schellino R.	Grant for internationalization (GFI)	Coordinator	15,000 €	UNITO
2022-2025	Cross-talk between intrinsic and extrinsic mechanism in importin alpha 3 knock-out mice. Investigating the neurite outgrowth in embryonic neurons and the	Marvaldi L.	Programma per Giovani Ricercatori "Rita Levi Montalcini"	Coordinator	251,581,44	UNITO

	control gene regulation in importin alpha3 knock-out mice.					

3. SCIENTIFIC ACTIVITIES IN 2022

Alessandro Vercelli, PI

Supervised PhD students:	A. Caretto (co-tutorship with M. Boido), G. Pavarino, S. Dallere
Honors, prizes, awards:	President Italian Society for Neuroscience, elected member Academy of Medicine of Torino, elected member of the Directive Committee of Italian Anatomists
Outreach activities	
• International collaborations:	G. Aumayr (Austria), M. Summers (Australia), S. Berlin (Technion, I)
• Invited talks:[99]	
• Science communication:[100]	
• Editorial duties:	Associate Editor Frontiers in Ageing, Editor in Anatomy, Editor in Chemical Neuroanatomy
• others[101]	
Organizational activities and responsibilities at NICO:	Scientific Director
Speakers invited:	Marco Tripodi (Cambridge, UK), Nir Giladi (Tel Aviv University, Israel), Stefano Suzzi (Weizmann Institute)
Other organizational activities:[102]	Deputy Rector for Biomedical Research, UNITO; Participation Governing Council IBRO and FENS; vice director for Research Department Neuroscience
Workshops, Schools or Conferences organized:	Italian-Israeli Neuroscience Societies Joint Symposium (Eilat)
Technology transfer achievements (patents, etc.):	President Spin-off committee, UNITO; UNITO representative in European Institute of Innovation & Technology Health

Marina Boido, Associate Professor[103]

Supervised PhD students:	A. Caretto (co-tutorship with A. Vercelli), F. Virla (co-tutorship with R. Mariotti, UNIVR), D.M. Rasà
Honors, prizes, awards:	na
Outreach activities	

[99] Invited seminars, invited talks at meeting and symposia, invitation upon selection at meetings
[100] Public engagement
[101] Posters at meetings, participation in the board of scientific societies, referee for grant agencies
[102] No university appointments.
[103] Please duplicate the module for the various lab members

• International collaborations:	Prof. Artero, Univ. Valencia; Prof. Soler, University of Lleida, Spain; Pharmafox Therapeutics AG, Switzerland; Dr. Martinat, I-STEM, Corbeil-Essonnes; P. Smeriglio, Institut de Myologie, Paris; A. Prochiantz, Collège de France, Paris; A.C. Cristovao, University of Beira Interior, Portugal
• Invited talks:	Boido M. Out-of-the-box therapeutic strategies for spinal muscular atrophy. ISFN 30th Annual meeting 2022, Joint Symposium of the Italian-Israeli Neuroscience Societies on Neurodegenerative Diseases. Eilat, Israel 04-06/12/2022. Boido M. Neuromuscular junctions: from development to aging and disease. XVI FISV Congress, Portici (Napoli), 14-16/09/2022.
• Science communication:	Giovedì Scienza, live streaming from NICO, "Vivere per sempre. Una popolazione sempre più longeva, i suoi problemi e le risposte della ricerca" (10/03/2022) Outreach conference "Un viaggio nell'atrofia spinale muscolare (SMA): dai pazienti ai ricercatori e ritorno", at Fondazione Caritro, Trento (11/03/2022) Notte Europea delle Ricercatrici e dei Ricercatori 2022 (UNIGHT, European Researchers night). "Into the Brain: connessioni che non ti aspetti: inquinamento, vivere green e cervello". Contribution to the exhibition "The mountain touch" at Museo Nazionale della Montagna, Turin (04/11/2022-02/04/2023) Video interview for Trend Sanità - Policy and Procurement in Health Care "Drug repositioning at the service of rare diseases" (21/11/2022) Regional coordinator (Piedmont) of Olympics in Neuroscience; Regional stage, Turin, 19/03/2022
• Editorial duties:	Review Editor for Frontiers in Aging Neuroscience and in Alzheimer's Disease and Related Dementias
• Others	General Secretary in the SIBS directive board Poster at meetings: Boido M, Stanga S, Pavarino G, Vercelli A. "Mitochondrial morpho-functional dysfunctions in spinal muscular atrophy" FENS 2022, 9-13.06.22 Paris, France.
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)", light sheet microscope

	Organization of the NICO NeuroWebinars
Speakers invited:	Stas Engel (Univ. Ben Gurion)
Other organizational activities:	CEO of S&P BRAIN SRL spinoff
Workshops, Schools or Conferences organized:	President of SIBS National Conference 2022 Organization of the II edition of the conference on: "Motor neuron diseases: understanding the pathogenetic mechanisms to develop therapies" with Dr. Stanga, Turin, 4-5/11/22
Technology transfer achievements (patents, etc.):	Co-inventor of the European Patent Application n. 21383162.1 filed on 20 December 2021 and PCT n. PCT/EP2022/087022 [by Università di Torino, Universitat de València, CECS, INSERM Transferet e Univeriste D'Evry – Val D'Esson (together with G. Menduti)

Corrado Calì, Assistant Professor RTD-B

Supervised PhD students:	Maria Fernanda Veloz Castillo (Co-Tutele with Pierre Magistretti, KAUST)
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	P. Magistretti (KAUST, Thuwal, Saudi Arabia); H. Pfister (Harvard, Cambridge, USA); P. Bezzi (UNIL, Lausanne Switzerland); E. De Schutter (OIST, Okinawa, Japan); J. F. Oliveira (UniMino, Braga, Portugal); Renaud Jolivet (Maastricht University)
• Invited talks:	Extreme-Scale representation and simulation of physiological structures, EMIx Workshop 2022 Simula Research Laboratory, Oslo (Norway), June 10. TERATEC Forum 2022 "HPC Technologies and Health" Ecole Polytechnique, Palezieux (France), June 15 CNS 2022 Glia Workshop "Emerging perspectives and models for Neuron-glial Interactions". Melbourne (Australia), July 19.
• Science communication:	Progetto "VICINI" (November 14th-18th); Ricercatori alla Spina (31.08; 9.11; 14.12); Life of a Neuron (2021 - 2023), Scientific - Artistic installation in Washington DC and New York (USA)
• Editorial duties:	Reviewer for Aging Cell; Guest Editor for Frontiers in Neuroscience
• Others	Permanent member of the BRAYN conference award revision panel; Member of the Review panel 45 for the ANR (Agence Nationale de la Recherche Francaise); SIBS representative at FISV directive board; Reviewer for EIC Pathfinder; Reviewer for the AU Research Grant, Ajman University, EAU

Organizational activities and responsibilities at NICO:	Outreach activity lead for Vercelli group
Speakers invited:	na
Other organizational activities:	Founder and President of IntraVides SRL
Workshops, Schools or Conferences organized:	President of SIBS National Conference 2022; Symposium at FENS Regional Meeting in Algarve (Portugal) on Computational Models of Glial Cells
Technology transfer achievements (patents, etc.):	SmartMoney, Innovation Manager, POR-FSE (rough total of 50k Euros worth) for the startup Intravides SRL

Letizia Marvaldi, Assistant Professor RTD-B

Supervised PhD students:	Amisha Parmar
Honors, prizes, awards:	Rita Levi Montalcini Fellowship (MIUR)
Outreach activities	
• International collaborations:	Dr. Franziska Rother, MDC Berlin
• Invited talks:	na
• Science communication:	na
• Editorial duties:	na
• others	Poster at meeting: Poster Presentation at the "Motor neuron diseases: understanding the pathogenetic mechanisms to develop therapies" with Pr. Marina Boido e Serena Stanga, Turin, 4-5/11/22.
Organizational activities and responsibilities at NICO:	na
Speakers invited:	Dr. Terenzio Marco, OIST Institute Okinawa Japan
Other organizational activities:	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	Fainzilber Mike and Marvaldi Letizia PCT / il2020 / 050801. Yeda research and development Co. Ltd.

Roberta Schellino, Assistant Professor RTD-B (since November 2022)

Supervised PhD students:	na
Honors, prizes, awards:	IBRO travel grant award to attend the "3rd International Scientific Congress on Spinal Muscular Atrophy (SMA Europe)", Barcelona, 21-23 October 2022
Outreach activities	
• International collaborations:	M. Parmar, Lund University; J. Willem Vrijbloed, Pharmafox Therapeutics AG; R. Fariello, Pharmafox Therapeutics AG
• Invited talks:	Schellino R, Menduti G, Boido M., Vercelli A. Cortical alterations in a murine model of SMA: focus on interneurons and projection neurons". 3rd International Scientific & Clinical Congress on Spinal Muscular Atrophy, SMA Europe. 21-23 October, 2022. Barcelona, Spain.

• Science communication:	Notte Europea delle Ricercatrici e dei Ricercatori 2022 (UNIGHT, European Researchers night). “Into the Brain: connessioni che non ti aspetti: inquinamento, vivere green e cervello”. Communication: “Il cervello in un bosco: vivere il verde come terapia non farmacologica e per prevenire le malattie neuropsichiatriche”. Neuroscience Institute Cavalieri Ottolenghi (NICO), Orbassano (TO). Symposium “Sinapsi _ Forum Neuroscienze”. Communication: “Trapianti di cellule staminali in un modello di malattia di Huntington”. Scuola di Studi Superiori Ferdinando Rossi (SSST), University of Turin. 22 September 2022. Member of local organizing committee at “Olimpiadi delle Neuroscienze”, Torino, 19/03/2022
• Editorial duties:	Review board for the journals: Brain Sciences (ISSN 2076-3425), MDPI group; The International Journal of Molecular Sciences (ISSN 1422-0067), MDPI group; Journal of Clinical Medicine (ISSN 2077-0383), MDPI group; Journal of Developmental Biology (ISSN 2221-3759), MDPI group.
• others	Oral presentation at meeting: Schellino R, Menduti G, Boido M, Vercelli A. “Cerebral cortex alterations in a SMA mouse model”. 32nd National Conference of the Italian Group for the Study of Neuromorphology “Gruppo Italiano per lo Studio della Neuromorfologia” G.I.S.N., Naples, November 25-26, 2022. Poster at meeting: Schellino R, Menduti G, Boido M, Vercelli A. “Cytoarchitecture of the cerebral cortex of a murine model of spinal muscular atrophy: focus on projection neurons and interneurons“. II Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies”. 4-5 November, Turin, Italy.
Organizational activities and responsibilities at NICO:	Responsible for E800 Nikon Eclipse fluorescence microscope
Speakers invited:	na
Other organizational activities:	Member of local organizing committee at SIBS 94th National Congress. Turin, 06-09/04/2022. Member of the local committee for: Workshop "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies” (II edition). Turin, November 04–05, 2022.
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Serena Stanga, Assistant Professor RTD-B

Supervised PhD students:	na
Honors, prizes, awards:	National Scientific qualification as associate, Academic Recruitment Field 05/H - Human anatomy and histology, according to the Italian higher education system, in the call 2021/2023 (Ministerial Decree n. 553/2021 and 589/2021) for the disciplinary field of 05/H1 - Human anatomy; Winner of the IBRO Collaborative Research Grant 2022: Award to spend a period as Visiting Scientist (3 months) at Cedars-Sinai, Los Angeles, California, USA.
Outreach activities	
• International collaborations:	P. Kienlen-Campard, Institute of Neuroscience, UCLouvain, Belgium; E. Audouard, Institut du Cerveau et de la Moelle Épinière INSERM, Paris, France; F. Piguet, Institut du Cerveau et de la Moelle Épinière INSERM, Paris, France; A. Prochiantz, Center for Interdisciplinary Research in Biology (CIRB), Collège de France, Paris; C. Svendsen, Board of Governors Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA; A.C. Cristovao, University of Beira Interior, Portugal.
• Invited talks:	"Activation of the hepcidin-ferroportin1 pathway in the brain and astrocytic-neuronal crosstalk to counteract iron dyshomeostasis during aging". Women in Neuroscience Symposium 2022 (WIN), Tbilisi, Georgia, 13/08/22; "Mitochondrial dysfunctions and iron dyshomeostasis: a red thread across aging and neurodegenerative diseases". Cedars-Sinai, Los Angeles, CA (USA), Svendsen lab, Board of Governors Regenerative Medicine Institute, 01/09/22.
• Science communication:	Pint of Science: "Braccio di ferro col cervello", 10/05/22 (Birrificio Torino)
• Editorial duties:	Review editor, 1 editorial contribution for Frontiers in Neuroscience.
• others	Scientific Expert for the scientific panel Interfaces of the National Research Agency (ANR), AAPG 2022; Evaluator for the Joint Programming Neurodegenerative Disease Research (JPND); Evaluator for the European Commission call HORIZON-MSCA-2022-DN-01; Posters at meetings: Mezzanotte M, Ammirata G, Boido M, Roetto A, Stanga S. "Activation of the hepcidin-ferroportin1 pathway in the

	brain and astrocytic-neuronal crosstalk to counteract iron dyshomeostasis during aging" SIBS 2022, 6-9.04.22, Torino; Stanga S, Mezzanotte M, Ammirata G, Boido M, Roetto A. "Activation of the hepcidin-ferroportin1 pathway in the brain and astrocytic-neuronal crosstalk to counteract iron dyshomeostasis during aging" FENS 2022, 9-13.06.22 Paris, France.
Organizational activities and responsibilities at NICO:	- Responsible for the Cell Culture room (floor 0) - Responsible for the dissection room (floor -1) - Responsible for the Green NICO Committee
Speakers invited:	Nicoletta Filigheddu, DIMET - Dipartimento di Medicina Traslazionale, Università del Piemonte Orientale, Novara, Italy
Other organizational activities:	Member of Organizational and Scientific Committee Member of the 94° meeting of the SIBS: Società Italiana di Biologia Sperimentale, Torino, 6-9/04/2022; Organization of the Lab Meetings 2022 of the group: Brain development & disease
Workshops, Schools or Conferences organized:	Organization of the II edition of the conference on: "Motor neuron diseases: understanding the pathogenetic mechanisms to develop therapies" with Pr. Marina Boido, Torino, 4-5/11/22.
Technology transfer achievements (patents, etc.):	na

Giovanna Menduti, Postdoc

Supervised PhD students:	na
Honors, prizes, awards:	"Young Investigator award" for the talk presentation at the "94th SIBS Congress" (Italian Society of Experimental Biology), April 6-9, 2022, Turin, in the congress session "Neurodegeneration and Neuroinflammation". Individual Research Fellowship (Assegno di Ricerca), Department of Neuroscience "Rita Levi Montalcini", University of Torino. Project title: "Il ruolo dell'asse GABA-mitocondri nell'Atrofia Muscolare Spinale".
Outreach activities	
• International collaborations:	na
• Invited talks:	na
• Science communication:	na
• Editorial duties:	na
• others	Oral presentation at meeting: "Moxifloxacin rescues Spinal Muscular Atrophy phenotypes in both animal model and patient-derived cells". Menduti G, Konieczny P, Januel C, Martinat C,

	Artero R, Boido M. 94th SIBS Congress (Italian Society of Experimental Biology), April 6-9, 2022, in Turin, Italy. Posters at meeting: "Drug repositioning strategy in Spinal Muscular Atrophy: therapeutic effects of the antibiotic Moxifloxacin in SMNΔ7 mice and in patient derived-cells". Menduti G, Konieczny P, Januel C, Martinat C, Artero R, Boido M. "GABA signaling and metabolism (dys)regulation in Spinal Muscular Atrophy". Menduti G, Beltrando G, Vercelli A, Boido M. BraYn - 5th Brainstorming Research Assembly for Young Neuroscientists. September 28-30, Rome, Italy. "Drug repositioning in Spinal Muscular Atrophy: therapeutic effects of the antibiotic Moxifloxacin in SMNΔ7 mice". Menduti G, Konieczny P, Januel C, Martinat C, Artero R, Boido M. 3rd International Scientific & Clinical Congress on Spinal Muscular Atrophy, SMA Europe. October 21-23, 2022. Barcelona, Spain.
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:	Member of the local committee for: Workshop "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies" (II edition). Turin, November 04–05, 2022. Member of local organizing committee at SIBS 94th National Congress. Turin, 06-09/04/2022.
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	Co-inventor of the European Patent Application n. 21383162.1 filed on 20 December 2021 and PCT n. PCT/EP2022/087022 [by Università di Torino, Universitat de València, CECS, INSERM Transferet e Univeriste D'Evry – Val D'Esson (together with M. Boido)

Mariarosa Mezzanotte, Postdoc

Supervised PhD students:	na
Honors, prizes, awards:	Individual Research Fellowship (Assegno di Ricerca), Department of Neuroscience "Rita Levi Montalcini", University of Turin. Project title: "Studio del metabolismo cerebrale del ferro e della funzionalità mitocondriale in modelli cellulari e murini di Malattia di Alzheimer"
Outreach activities	
• International collaborations:	Pr. Maja Vujic Spasic, Institute for Molecular Endocrinology of Animals, Ulm, Germany.

• Invited talks:	na
• Science communication:	Support in the organization and sharing of research through practical laboratory initiative of the "Notte Europea dei Ricercatori" Neuroscience Institute Cavalieri Ottolenghi (NICO), Turin, 30/09/2022.
• Editorial duties:	na
• Others	Poster at meeting: Mezzanotte M*, Pavarino G*, Stanga S, Vercelli A, Boido M. "Aconitase inactivation and iron perturbation in the spinal cord of smaΔ7 mouse model" Motor Neuron Diseases (MND), Turin, 4-5/11/2022.
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:	Member of the local committee for: Workshop "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies" (II edition). Turin, November 04–05, 2022.
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Anna Caretto, PhD Student

Supervised PhD students:	na
Honors, prizes, awards:	"Prize for young researchers" for the oral communication "Glycinergic system alterations in SMA". 32nd National Conference of the Italian Group for the Study of Neuromorphology (GISN). November, 25th-26th 2022, Naples. "Young Investigator Award" for the poster "Targeting of altered mitochondrial genes as a therapeutic strategy for Spinal Muscular Atrophy" al 94th SIBS National Congress. April, 6th-9th 2022, Turin.
Outreach activities	
• International collaborations:	na
• Invited talks:	na
• Science communication:	Member of local organizing committee at "Olimpiadi delle Neuroscienze", Turin, 19/03/2022
• Editorial duties:	na
• others	Oral presentation at meeting: Caretto A, Di Cunto F, Boido M, Vercelli A. Glycinergic system alterations in SMA. 32nd National Conference of the Italian Group for the Study of Neuromorphology (GISN). November, 25th-26th 2022, Naples. Posters at meeting:

	Caretto A, Di Cunto F, Boido M, Vercelli A. Mitochondrial SMN1-anticorrelated gene analysis sheds light on possible glycinergic system alterations in Spinal Muscular Atrophy. Motor Neuron Disease: understanding the pathogenetic mechanisms to develop therapies (2nd Edition). November 4th-5th, Turin. Caretto A, Gesmundo I, Schellino R, Schally AV, Granata R, Boido M, Vercelli A. Investigating the GHRH agonist MR409 therapeutic role in a mouse model of Spinal Muscular Atrophy. 3rd International Scientific Congress on Spinal Muscular Atrophy (SMA Europe 2022). October 21st-23rd, Barcelona. Caretto A, Di Cunto F, Boido M, Vercelli A. Mitochondrial SMN1-anticorrelated genes as potential targets for Spinal Muscular Atrophy therapy. Fifth brainstorming research assembly for young neuroscientists (BraYn 2022). September, 28th-30th 2022, Rome. Caretto A, Di Cunto F, Boido M, Vercelli A. Drug repositioning as a therapeutic strategy to target mitochondria in Spinal Muscular Atrophy. ENCODS2022. July, 7th-8th 2022, Paris. Caretto A, Di Cunto F, Boido M, Vercelli A. Investigation of mitochondrial SMN1-anticorrelated genes as possible therapeutic targets for Spinal Muscular Atrophy. National meeting of PhD Students in Neuroscience. June 11th 2022, Brescia. Caretto A, Di Cunto F, Boido M, Vercelli A. Targeting of altered mitochondrial genes as a therapeutic strategy for Spinal Muscular Atrophy. SIBS 94th National Congress. April, 6th-9th 2022, Turin.
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:	Member of local organizing committee at Motor Neuron Disease: understanding the pathogenetic mechanisms to develop therapies (2nd Edition). Turin, 04-05/11/2022. Member of local organizing committee at SIBS 94th National Congress. Turin, 06-09/04/2022.
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Sveva Dallere, PhD Student

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	Pharmafox Therapeutics AG
• Invited talks:	na
• Science communication:	Member of local organizing committee at "Olimpiadi delle Neuroscienze", Torino, 19/03/2022
• Editorial duties:	na
• others	na
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:	Member of local organizing committee at SIBS 94th National Congress. Turin, 06-09/04/2022. Member of local organizing committee at Motor Neuron Disease: understanding the pathogenetic mechanisms to develop therapies (2nd Edition). Turin, 04-05/11/2022.
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Gianna Pavarino, PhD student

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	na
• Invited talks:	na
• Science communication:	Poster preparation for the dissemination event "U*NIGHT - La Notte Europea dei Ricercatori e delle Ricercatrici 2022 The Green Brain: un "Caffe Scientifico" per comprendere l'impatto di alimenti e inquinanti ambientali sulla salute del nostro cervello". Turin, October 1, 2022
• Editorial duties:	na
• others	Posters at meeting: Mezzanotte M*, Pavarino G*, Stanga S, Vercelli A, Boido M. "Aconitase inactivation and iron perturbation in the spinal cord of SMAΔ7 mouse model". Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies (2nd edition). Turin, November 4-5, 2022 Pavarino G, Stanga S, Zummo PF, Vercelli A, Boido M. "Mitochondrial dysfunctions in Spinal Muscular Atrophy: mitochondrial aconitase as a potential biomarker of the disease". 5th Brainstorming Research Assemply for Young Neuroscientists (BraYn). Rome, September 28-30, 2022.

	Pavarino G*, Zummo PF*, Stanga S, Vercelli A, Boido M. "The study of mitochondrial morpho-functional dysfunctions in the pathogenesis of spinal muscular atrophy reveales mitochondrial aconitase as a possible biomarker of the disease". 94th National Congress of the Italian Society of Experimental Biology. Turin, April 06-09, 2022.
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:	Member of local organizing committee at Motor Neuron Disease: understanding the pathogenetic mechanisms to develop therapies (2nd Edition). Turin, 04-05/11/2022. Member of local organizing committee at SIBS 94th National Congress. Turin, 06-09/04/2022.
Workshops, Schools or Conferences organized:	Practical workshop on how to use MiNA (Mitochondrial Network Analysis) plugin of ImageJ at NICO held the 2nd day of the Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies (2nd edition). Turin, November 4-5, 2022
Technology transfer achievements (patents, etc.):	na

Daniela Maria Rasà, PhD student

Supervised PhD students:	na
Honors, prizes, awards:	"Young Investigator Award" for the poster "2D and 3D in vitro experimental models to study spinal muscular atrophy and preliminary perform drug screening" (Daniela Maria Rasà, Serena Stanga, Marina Boido, Alessandro Vercelli), 94° Congresso Nazionale della Società Italiana di Biologia Sperimentale, Turin, 06-09/04/2022
Outreach activities	
• International collaborations:	na
• Invited talks:	"Cortical neuron and organotypic spinal cord cultures: valuable experimental 2D and 3D models of SMA", NMJ in a dish workshop, SMA Europe, Barcelona, 21-23/10/2022
• Science communication:	Member of local organizing committee at "Olimpiadi delle Neuroscienze", Turin, 19/03/2022
• Editorial duties:	na
• others	Posters at meeting: "2D and 3D *in vitro* experimental models to study spinal muscular atrophy and preliminary perform drug screening" (Daniela Maria Rasà, Serena Stanga, Marina Boido, Alessandro Vercelli), 94° Congresso Nazionale della Società Italiana di Biologia Sperimentale, Turin, 06-09/04/2022

	"Unravelling the effect of stressors on Amyotrophic Lateral Sclerosis onset and progression: preliminary in vitro experiments" (Daniela Maria Rasà, Marina Boido), New Perspectives in Neuroscience: Research Results of Young Italian Neuroscientists, Brescia, 11/06/2022 "New SMN-independent drugs for Spinal Muscular Atrophy treatment" (Daniela Maria Rasà, Serena Stanga, Pamela Santonicola, Marina Boido, Elia Di Schiavi, Alessandro Vercelli), ENCODS 2022, Paris, 07-08/07/ 2022 "A preliminary *in vitro* study to assess the stressor effect on Amyotrophic Lateral Sclerosis onset and progression" (Daniela Maria Rasà, Ilaria Stoppa, Marina Boido), 5th BRAYN, Rome, 28-30/09/2022 "Cortical neuron and organotypic spinal cord cultures: valuable experimental 2D and 3D models of SMA" (Daniela Maria Rasà, Serena Stanga, Marina Boido, Alessandro Vercelli), SMA Europe, Barcelona, 21-23/10/2022 "An *in vitro* set-up study to assess stressor effect on amyotrophic lateral sclerosis onset and progression" (Daniela Maria Rasà, Ilaria Stoppa, Marina Boido), II Edition of Motor Neuron Diseases, Turin, 04-05/11/2022
Organizational activities and responsibilities at NICO:	Member of green committee
Speakers invited:	na
Other organizational activities:	Member of local organizing committee at SIBS 94th National Congress. Turin, 06-09/04/2022. Member of local organizing committee at Motor Neuron Disease: understanding the pathogenetic mechanisms to develop therapies (2nd Edition). Turin, 04-05/11/2022.
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Silvia de la Morena, Fellowship recipient

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	na
• Invited talks:	na
• Science communication:	Member of local organizing committee at "Olimpiadi delle Neuroscienze", Turin, 19/03/2022

• Editorial duties:	na
• others	na
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:	Member of local organizing committee at SIBS 94th National Congress. Turin, 06-09/04/2022.
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Cristina Ruatti, Fellowship recipient

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	na
• Invited talks:	na
• Science communication:	Member of local organizing committee at "Olimpiadi delle Neuroscienze", Turin, 19/03/2022
• Editorial duties:	na
• others	na
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:	Member of local organizing committee at Motor Neuron Disease: understanding the pathogenetic mechanisms to develop therapies (2nd Edition). Turin, 04-05/11/2022. Member of local organizing committee at SIBS 94th National Congress. Turin, 06-09/04/2022.
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Vanessa Chiappini, Fellowship recipient

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
● International collaborations:	na
● Invited talks:	na
● Science communication:	na
● Editorial duties:	na
● others	Poster at meeting: Chiappini V, Traldi C, Tonda Turo C, Boido M. A 3D bioprinted model of spinal cord: possible applications in the

	MN disease field. Motor Neuron Disease: understanding the pathogenetic mechanisms to develop therapies (2nd Edition). Turin, 04-05/11/2022.
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:	Member of local organizing committee at Motor Neuron Disease: understanding the pathogenetic mechanisms to develop therapies (2nd Edition). Turin, 04-05/11/2022. Member of local organizing committee at SIBS 94th National Congress. Turin, 06-09-/04/2022.
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

ALL LAB MEMBERS

Activities:[104]	na

4. Research activity in 2022[105]

a. Summary (500 characters)

We study CNS development and the neurobiological mechanisms and molecular pathways leading to normal development and neurodegeneration. We are interested in neuronal cell death pathways (in development and in neurodegeneration) 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 and ALS. We are also interested in pain signaling and neuronal regeneration.

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 on brain function, disease and repair. Understanding the

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

CNS development and how neurons establish synaptic connections and create networks is key to the comprehension of brain function and disease, and to design new therapeutic strategies. To this regard, astrocytes participate in tuning neuronal network at various levels (structural, metabolic, chemical), to be fully understood. To explore these aspects, we take advantage of normal brains and compare with TG mice models, 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 both foster a translation from bench to bedside, and 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 cross-breed neuroscience with other disciplines, with a focus on technological improvement, 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, 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 also intend to understand the communication between intrinsic and extrinsic factors in sensory neurons.
We study the neuronal death mechanisms during development and disease, the neuroinflammation and how to prevent it.
Stem cells (SCs) are still a growing field of research: we use neural and/or mesenchymal SCs to treat neurodegenerative/traumatic diseases (in ALS and SCI), to provide trophic and immunomodulatory factors to host neurons. We are also interested in the effect of (green) environment on mental health and neurodegenerative diseases.

d. Results (4000 characters)

Astrocytes-neurons structural crosstalk
CC is working on a high-resolution EM dataset from a human sample that has been used to segment and extract the morphology of one neuron and the mitochondria (see https://www.artechouse.com/program/life-of-a-neuron/). We are working on this dataset, as well as a recently published human dataset from the group of Jeff Lichtmann, to investigate the ultrastructural relationships among astrocytes and neurons, using computational tools developed in collaboration with the Hanspeter Pfister group (Harvard).
We are now working on using light-sheet microscopy to highlight possible arrangements between astrocytes and dendritic bundles on the whole brain, to highlight microdomains of interest to investigate using 3DEM.

Altered cellular and molecular mechanisms in SMA and therapeutic approaches
MB, SS & AV are studying mitochondria impairment in SMA: in addition to murine samples, mitochondrial alterations have been also evaluated in SMA patients-derived fibroblasts (paper in preparation).
MB&AV (and RS, GM) are looking at the cerebral cortex of SMA mice at different postnatal ages, to investigate alterations (in number and morphology) in projection neurons and interneurons, both in the motor and somatosensory cortex: we also observed an impairment of cortical GABAergic neurotransmission in SMA mice. Moreover, in the spinal cord we (AC) detected an impairment of the glycinergic degradation system (alterations in *GCSH* expression and Renshaw cells morphology).
With G. Viero (CNR, Trento) MB (with GP and CR) are deepening the SMN functions, analyzing the presence of translation defects in our SMA mice, and understanding the interaction of SMN with cell organelles.
MB&AV are also testing repositioned SMN-dependent (in coll with Univ. Valencia; GM, CR) and -independent drugs (in coll with CNR, Naples; DMR, SS) and other therapeutic approaches (a GHRH agonist and MSC-derived exosomes) to delay disease progression and counteract muscular atrophy: all the approaches

assured positive outcomes, counteracting neurodegeneration, neuroinflammation and/or muscular atrophy. We recently published some of these data.

Stem cell therapy in HD and ALS
With E. Cattaneo and A. Buffo, MB (and RS, SD) evaluated the effects of long-term (6 months) transplantation of human embryonic stem cells and enriched environment (EE) on striatal circuit reconstruction in a rat model of HD (submitted).
Regarding ALS, in collaboration with A. Vescovi, MB (with SdM, DMR) is testing the therapeutic potential of clinical-grade human neural stem cells in the SOD1 G93A mouse model: at now, survived cells have been observed only at early time-points, but we are testing different experimental options.

Spinal cord injury
In collaboration with Dr Tonda-Turo (Polytechnic of Turin), MB (and VC) are developing a 3D cellularized construct by bioprinting technique, to preliminarily screen treatments for SCI. We are defining the conditions to co-culture neurons and glial cells in 3D, and performing the first bioprinting experiments.

Active and Healthy Ageing
AV, MB, RS, GP and SD are investigating the effect of natural compound (terpenes) exposure on mental health affected patients and on cellular and animal models of neurodegenerative diseases (e.g. Alzheimer disease).
Moreover, SS, MM and MB clarified the mechanism of brain iron accumulation during aging by demonstrating that canonical iron regulators, mainly expressed in the liver, are active also in the brain as response to a higher brain iron influx in order to control brain iron homeostasis
Finally, MB&DMR are evaluating how a stressful lifestyle can impact on the onset and progression of ALS: preliminary in vitro results suggest an increased susceptibility to stressors for SOD1 G93A cells.

e. Advancement in the field (1000 characters)

Our group works in several hot topics in Neuroscience, such as axonal development/growth in brain physiology and pathology, study of cell complexity and interplay through 3D models, cell death and therapy. It is also involved in the study of anatomical/functional connectivity of the human brain, and how it is altered in disease. In 2022, we have obtained significant results in the field of SMA and motoneuron diseases, by identifying new drugs which extend the lifespan of the animal models of disease and reduce its effects. These data will be published in early 2023 in several papers. In particular, the identification of an agonist of GHRH as a protective molecule and the identification of new molecules preventing muscular atrophy by acting on the neuromuscular junction will represent some milestones in our work.
The ongoing projects on Digital and biological twin of the patient in the D34H will allow to change the paradigms in precision and predictive medicine.

f. Publications[106]

Abdellah M, Cantero JJG, Guerrero NR, Foni A, Coggan JS, **Calì C**, Agus M, Zisis E, Keller D, Hadwiger M, Magistretti PJ, Markram H, Schürmann, F. 2023 Ultraliser: a framework for creating multiscale, high-fidelity and geometrically realistic 3D models for in silico neuroscience. Briefings in Bioinformatics 24(1): bbac491.

Agus M, Aboulhassan A, Al Thelaya K, Pintore G, Gobbetti E, **Calì C**, Schneider J. 2022 Volume Puzzle: visual analysis of segmented volume data with multivariate attributes. IEEE Visualization and Visual Analytics (VIS), Oklahoma City, OK, USA 130-134.

Boido M, **Vercelli A**. 2022 "Spinal cord injury", chapter in the book "Neurobiology of Brain Disorders II edition", published by Zigmond MJ, Wiley CA, Chesselet M-F. Elsevier New York. ISBN 9780323856546, p. 427–441.

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

Bosso T, Vischia F, Keller R, Vai D, Imperiale D, **Vercelli A**. A case report and literature review of cognitive malingering and psychopathology. Front Psychiatry. 2022 Oct 14;13:981475.

Calì C, Nuzzolese E. 2022. The use of the Anatomage Table for improving forensic odontology education and training. Annals of 3D Printed Medicine 7, 100073.

Canciani A, Capitanio C, **Stanga S**, Faravelli S, Scietti L, Mapelli L, Soda T, D'Angelo E, Kienlen-Campard P, Forneris F. 2022 Deconstruction of Neurotrypsin Reveals a Multi-factorially Regulated Activity Affecting Myotube Formation and Neuronal Excitability. Mol Neurobiol. Dec;59(12):7466-7485.

De Oliveira Figueiredo EC, **Calì C**, Petrelli F, Bezzi P. 2022. Emerging evidence for astrocyte dysfunction in schizophrenia. Glia 70 (9), 1585-1604

Januel C*, **Menduti G***, Mamchaoui K, Martinat C, Artero R, Konieczny P, **Boido M**. Moxifloxacin rescues SMA phenotypes in patient-derived cells and animal model. Cell Mol Life Sci. 2022 Jul 22;79(8):441.

Mezzanotte M, Ammirata G, **Boido M**, **Stanga S***, Roetto A*. 2022 Activation of the Hepcidin-Ferroportin1 pathway in the brain and astrocytic-neuronal crosstalk to counteract iron dyshomeostasis during aging. Sci Rep. Jul 9;12(1):11724.

Roveta F, Cermelli A, Boschi S, Ferrandes F, Grassini A, Marcinnò A, Spina M, Rubino E, Borsello T, **Vercelli A**, Rainero I. Synaptic Proteins as Fluid Biomarkers in Alzheimer's Disease: A Systematic Review and Meta-Analysis. J Alzheimers Dis. 2022;90(4):1381-1393.

Troidl J, **Calì C**, Gröller E, Pfister H, Hadwiger M, Beyer J. 2022. Barrio: Customizable Spatial Neighborhood Analysis and Comparison for Nanoscale Brain Structures. Computer Graphics Forum. 41: 183-194.

Wyart E, Y Hsu M, Sartori R, Mina E, Rausch V, Pierobon ES, **Mezzanotte M**, Pezzini C, Bindels LB, Lauria A, Penna F, Hirsch E, Martini M, Mazzone M, Roetto A, Geninatti Crich S, Prenen H, Sandri M, Menga A, Porporato PE. 2022 Iron supplementation is sufficient to rescue skeletal muscle mass and function in cancer cachexia. EMBO Rep. Apr 5;23(4):e53746.

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 exploit our previous research on i) axonal growth in the CNS, ii) the astrocytic morphology and their interplay with neurons, iii) mechanisms of neuronal death in neurodegenerative and age-associated diseases, iv) multiscale network analysis v) stem cell therapy. We aim at investigating how urban green environmental spaces and natural compound inhalation affect mental health and can counteract neurodegeneration. We aim at investigating how astrocytic morphology relates in particular to dendritic bundles, whose activity might be coordinated by ensheating astroglial cells. There is also a growing interest on mitochondria in neurodegenerative diseases that we are extending to several pathologies (SMA, PD and AD). We aim at identifying some new therapeutic targets for traumatic and neurodegenerative diseases (as SCI, SMA, ALS and HD). By investigating the role of mitochondrial-iron metabolism in AD, we also aim to understand why elderly people present systemic anemia but accumulate iron in the CNS, a feature that is also common to many neurodegenerative diseases: we plan to study the mechanisms responsible for age-dependent brain iron increase and its potential involvement in the neurodegenerative processes in AD. We also intend to investigate the PNS, by looking at pain signaling and the neurogenetics of pain: moreover we have established DRG

culture in embryos, adult and aged animals to monitor neuronal growth assay and neuronal survival upon neurotrophin stimulations in importin alpha 3 ko mice.
We are importing new techniques, such as iPSCs and organoids for *in vitro* analysis of brain development and disease modeling, light sheet microscopy and innovative clearing protocols, 3D EM, and semi-automated tool for 3D analysis in VR.
AV is coordinating the UNITO researchers involved in the PNRR project D34H, which aims to build a digital and biological twin of the patient, by analysing clinica dataset obtained in different hospital in Italy to predict the evolution of a disease at his entrance at the hospital. Meanwhile, we will create biological models in vitro from IPSCs, organoids and assembloids from patient, to identify new markers of disease and to perform drug discovery. This project is related to personalized and precision medicine.

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. Considering the enormous economic and social impacts, finding a cure for neurodegenerative disorders remains a priority in science. Researchers are focused on identifying the common pathogenic processes shared among these diseases, in order to design new treatments and/or drug combinations and repurposing.
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, and understanding the co-occurrence and overall interactions among these diseases is the first step for drug development. These strategies therefore may be at a genetic, molecular, cellular and behavioral level, and must be considered in a holistic strategy. The majority of neurodegenerative disorders have significant genetic components, with genetic heritability such as for AD, ALS, HD and SMA that we largely study within our group.
To this aim we will collaborate with F. Di Cunto 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), P. Rocca (Turin, Schizophrenia and other psychiatric disorders) 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.
Moreover, the fine-tuning of synaptic signaling and brain-energy metabolism is another key process and hot topic in the CNS study. The fact that neurons express the machinery allowing them to self-sustain their basic functions is counterintuitive with respect to the assumption that astrocytes undergo a plethora of supporting roles for neurons, importantly metabolic support and fine tuning of synaptic transmission via gliotransmission, two faces of the same coin. The high spatial compartmentalization of astrocytes might be the key to solve such complex interplay between the two. Recently we have shown that glycogen, a mechanism of energy storage in astrocytes, is strategically located around synapses and large dendrites containing long mitochondrial bundles. Glycogen stores can be mobilized and used upon activation of astrocytic NA or VIP receptors. Work in the late 80s have already described the presence of VIP and NA fibers targeting L2/3 dendrites in visual cortex, where they arrange in bundles corresponding to cell bodies of L5 pyramidal neurons projecting to common targets. We are currently exploring whether the activity of these 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 are exploring 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: 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 Institute mission.

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

Spinal cord injury
SCI: in collaboration with Dr Tonda-Turo (Polytechnic of Turin), MB&VC are developing a 3D cellularized construct by bioprinting technique to 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.

Astrocyte-neuron interplay
Following our first results using iDISCO protocol, we intend to further improve the quality of the staining and investigate and eventually develop novel analytical strategies, considering our expertise in Volume Microscopy via 3DEM, that we can easily translate to similar data structures. CC&AV will focus on 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. To this regard, we recently obtained a custom-made glycogen antibody from Tsukuba University (Tokyo, Japan) with whom we will start collaborating. Quantitative data extracted from analysis on these samples will be used as input on a recently developed computational model in collaboration with OIST (Okinawa Institute of Science and Technology) to simulate calcium waves in real astrocytic morphology, previously obtained by our group.

Altered cellular and molecular mechanisms in motor neuron diseases and therapeutic approaches
SMA: MB, SS & AV (with GM) will extend our studies on GABA signaling and interneuron functionality, and their possible correlation in mitochondrial dynamics in SMA. With RS, we will further highlight the impaired neurotransmission and the functional alterations in SMA cortical networks that may contribute to disease pathology.
Moreover, thanks to two international grants (MB), we will further test both SMN-dependent and -independent approaches for SMA, in particular testing in vitro and in vivo repurposed drugs, also in combination with gene therapy. Additionally, we will elucidate if glycinergic degradation system is impaired in SMA, by exploiting new techniques, as RNAscope in situ hybridization.
ALS: MB (and DMR) will deepen the ongoing studies, by evaluating also in vivo how different stressors could negatively influence the onset and progression of ALS.

Aging
SS (and MM) will study the molecular and cellular mechanisms in age-associated neurodegeneration and role of mitochondrial-iron metabolism in AD: by using cellular and animal models, we will evaluate the levels of the iron pool available for intracellular metabolic reactions in the brain and their possible implications in determining cytotoxic effects and the decline in cognitive and motor skills. The effects of diets differently enriched in iron will be investigated in mice and advanced imaging techniques will be used to follow how mitochondria, iron and amyloid deposits interact in the brain.
MB, AV, RS (and SD) will also study the muscle innervation in elderly: in collaboration with Pharmafox Therapeutics AG, we will test different compounds able to support innervation and induce a limited hypertrophy of the muscles.

Mental health and urban green

In collaboration with Prof. Rocca (Univ. Torino) and Francesca Cirulli (Italian National Inst of Health), MB, AV, RS (and GP, SD) will study the effects of living in the green (close to city parks) on depression, schizophrenia and on neurodegenerative diseases (e.g. Alzheimer disease), 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.

Neuropathic pain
We are interested in how neuropathic pain is modulates by gender, aging, social interaction and rare disease. Research into these interesting interaction Will unlock novel approaches to personalized pain therapy.

Covid and CNS
We will study the Covid effects on CNS, by reproducing a cytokine storms on iPSC-derived neurons: we will study early cellular changes, including accumulation of neurofilaments and misaggregated proteins, signs of cell death and oxidative stress.

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

Some of our i) research topics, ii) methodologies employed and iii) external collaborations with top institutes, scientists and biotech companies, allow us to be involved in hot topics of research. Our studies on axonal thickness and its plasticity depending on the neuronal pattern of origin/projection, and on activity represent a new field which may have very important significance not only for normal development but also for disease.
Our experience on some molecular pathways related to neuronal death, such as JNK and those related to autophagy, is a specific competence which allowed us to design and test new therapeutic drugs. Moreover, the current collaborations (with Pharmafox, Naples CNR and Univ, Valencia) will give us the opportunity to patent some of the tested treatments.
The unique feature and ultimate goal of studying the mechanisms in age-associated neurodegeneration is to identify systemic biomarkers, prognostic of the cerebral iron status that may be predictive of cognitive impairment. An analysis of these potential markers will be conducted at the clinical level on elderly populations characterized by cognitive impairment.
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; Pierre Magistretti, Markus Hadwiger) and Hamad Bin Khalifa University (Qatar; Marco Agus). Recent microscopes are now acquiring bigger and bigger datasets (in the range of the Tera, if not Petabytes), and to this aim we are exploring new analytical strategies using newly developed quantum computing techniques, made available on cloud (e.g., IONQ).

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), if funded, will allow to design biosensors and lab-on-chip to the detection of biomarkers. MB&AV plan to use of human brain organoids derived from iPSCs, to study the CNS development and to “mimic” model of neurodegenerative diseases.
SS & AV will combine advanced *in vitro* and *ex vivo* techniques in order to study mitochondrial dynamics during ageing: with live imaging, we will trace and reconstruct mitochondrial networks, and by 2PM on organotypic cultures of brain slices of mice models we will investigate mitochondrial dysfunctions related to the amyloid pathology.
The recent installation of the light sheet microscope is pushing many teams to explore the tissue clearing protocol: iDISCO clearing and light sheet microscopy is being used to generate 3D volume visualizations of whole brains, showing structural astrocytes/neurons interplay (CC), as well as the alterations in cortical architecture and in neuronal projections of SMA animals compared to their WT littermates, at different

postnatal ages (MB, RS and AC). 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 are exploiting STED microscopy, to evaluate localization and interactions of SMN protein.
The collaboration with the group of prof. Cauda (Psychology Dept.) will allow using voxel-based morphometry, fMRI and tractography to study human anatomical and functional connectivity, and structure of the brain in ageing subjects. A collaboration is also under discussion to develop a neuroinformatic approach in studies of neurodegenerative diseases with F. Di Cunto and P. Provero.

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2022

Laboratory name: Clinical Neurobiology

1. LABORATORY DESCRIPTION – PERSONNEL:[107]

Principal Investigator

1) Capobianco, Marco Alfonso
   Position: Head ad interim of the Regional Reference Center for Multiple Sclerosis (CRESM) until June 2022
   Degree: MD
   Birthdate: 19/09/1972
   Phone: 011 9026 697
   Email: mcapobianco1972@gmail.com

Personnel

1) Sala, Arianna
   Position: Resident Medical biologist at the SCDO- Neurologia- AUO S. Luigi- Orbassano
   Degree: MSc, and Board Certification
   Birthdate: 22/05/1972
   Phone: 011 670 6601
   Email: sala.arianna72@gmail.com
   Role & expertise: CSF analysis, diagnostic/prognostic tests for MS and NMO, drug immunogenicity

2) Valentino, Paola
   Position: Medical biotechnologist
   Degree: MSc, and Board Certification
   Birthdate: 11/08/1981
   Phone: 011 670 6635
   Email: paolaval81@hotmail.com
   Role & expertise: biomarkers studies, management of biological material and quality system in CRESM Biobank

3) Montarolo, Francesca
   Position: post-doc (Research fellow at the Dept. of Neurosciences "Rita Levi Montalcini" in collaboration with the Laboratory of Neurophysiology of neurodegenerative diseases directed by Prof. Filippo Tempia.)
   Degree: MSc and PhD
   Birthdate: 14/05/1983
   Phone: 011 670 6632
   Email: francesca.montarolo@unito.it
   Role & expertise: Experimental and behavioral murine model studies, histological and molecular analyses

4) Martire, Serena
   Position: Medical Biotechnologist and Biostatistician
   Degree: MSc, and Board Certification
   Birthdate: 01/08/1987
   Phone: 011 670 6600

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

Email: serena.martire@unito.it
Role & expertise: Design and conduct of epidemiological and experimental studies, data analysis

5) Bava, Cecilia Irene
Position: Molecular Biotechnologist
Degree: MSc
Birthdate: 25/11/1996
Phone: 011 670 6635
Email: cecilia.bava@edu.unito.it
Role & expertise: sample processing and storage in CRESM Biobank, diagnostic tests for MS patients

6) Giorgi, Lucia
Position: Cellular and Molecular Biologist
Degree: MSc
Birthdate: 12/06/1995
Phone: 011 670 6635
Email: lucy.giorgi@gmail.com
Role & expertise: diagnostic tests for MS patients, sample processing and storage in CRESM Biobank

7) Bertolotto, Antonio
Position: Voluntary visitor (expert in MS)
Degree: MD
Birthdate: 12/02/1952
Phone: 011 670 66 00
Email: antonio.bertolotto@gmail.com
Role & expertise: Head of the Regional Reference Center for Multiple Sclerosis (CRESM) until 01/04/2021

2. CURRENT GRANTS

Starting-end date	Project Title and ID	Beneficiary[108]	Funding Program/Agency	Role of the unit[109]	Overall Amount Funded	Managed by FCO/UNITO
11/06/2021-31/12/2022	Implementazione del dosaggio dei neurofilamenti sierici a catena leggera (sNFL) nella pratica clinica in pazienti affetti da sclerosi multipla.	Dr.Capobianco	Roche	PI	39000	Yes
21/02/2022-31/12/2022	Approccio integrato per una efficace presa in carico post-emergenza COVID19 dei pazienti affetti da Sm	Dr. Capobianco	Novartis Farma spa	PI	36600	Yes
2021-2022	Neurofilamenti liquorali e fattori di evoluzione della Sclerosi Multipla	Dr. Capobianco	Università degli Studi di Catania	PI	16445	Yes
10/2022-09/2023	Biomarcatori di Neurodegenerazione nei pazienti con Sclerosi Multipla e NMOSD: studio pilota di applicazione del dosaggio dei neurofilamenti sierici nella pratica clinica corrente	-	AOU San Luigi Gonzaga	-	90000	Yes

3. SCIENTIFIC ACTIVITIES IN 2022

Marco Alfonso Capobianco

Supervised PhD students:	NA
Honors, prizes, awards:	NA
Outreach activities	
• International collaborations:	
• Invited talks:[110]	• Sclerosi Multipla le nuove terapie. Le malattie infettive tra innovazione e tradizione. Cuneo 13 dicembre 2022

[108] 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.
[109] Coordinator/PI of research unit/team component.
[110]Invited seminars, invited talks at meeting and symposia, invitation upon selection at meetings

	• Ofatumumab: dalle evidenze scientifiche all'impatto sulla pratica clinica. Konstellation. Milano 16 novembre 2022 • Scenario terapeutico attuale e futuro. Corner expert meeting focus su NMOSD. Genova 14 novembre 2022 • Clinical remarks: progression beyond relapses. Top podcasts in multiple sclerosis. Napoli 14 ottobre 2022 • Malattie demielinizzanti: Sclerosi Multipla o malattie NMO spectrum? JAMS. Torino 22-24 settembre 2022 • Long term efficacy and safety. ECHO in MS. Roma 15 giugno 2022 • Switching strategies - urgente combinazione di efficacia e sicurezza. AllinOne. Roma 25 maggio 2022 • MS mimics: MOGAD. XXIV approfondimenti monotematici sulla sclerosi multipla. Gallarate 25 marzo 2022 • NMOSD e MOGAD. Clinical Neuroimmunology. Napoli 10-11 febbraio 2022
• Science communication:[111]	Sclerosi Multipla e gravidanza. Settimana della salute della donna, fondazione ONDA. 22 aprile 2022
• Editorial duties:	Associate Editor for Frontiers Neurology, Journal of Neurology
• others[112]	Poster at ECTRIMS 2022 38th Congress of ECTRIMS, 27th Annual Conference of RIMS, Amsterdam, The Netherlands:
Organizational activities and responsibilities at NICO:	Ad interim PI Clinical Neurobiology Lab
Speakers invited:	• NMOSD e MOGAD. Corso di aggiornamento FAD su Sclerosi Multipla pediatrica di FISM • AHSCT come opzione terapeutica. Corso di aggiornamento FAD su Trapianto autologo di cellule staminali emolinfopoietiche nella sclerosi Multipla di FISM
Other organizational activities:[113]	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Arianna Sala, Resident Biologist

[111] Public engagement
[112] Posters at meetings, participation in the board of scientific societies, referee for grant agencies
[113] No university appointments.

Supervised PhD students:	NA
Honors, prizes, awards:	NA
Outreach activities	
• International collaborations:	NA
• Invited talks:	NA
• Science communication:	NA
• Editorial duties:	NA
• others	Partecipazione controlli di qualità di diagnostica di laboratorio nazionali ed internazionali quali • UK NEQAS, sezione di immunologia, per immunoisoelettrofocusing AINI (Associazione Italiana Neuroimmunologia) EQAS per anticorpi anti MOG, anti AQP4 e immunoisoelettrofocusing
Organizational activities and responsibilities at NICO:	NA
Speakers invited:	NA
Other organizational activities:	NA
Workshops, Schools or Conferences organized:	• "Comparison between live cell-based Fluorescence Activated Cell Sorting methodologies for diagnostic anti-MOG Ig detection"; 5° European NMOSD/MOGAD meeting (Lyon, 2-4/06/2022) • "2° corso di Diagnostica di laboratorio delle encefaliti autoimmuni e sindromi paraneoplastiche" Trento- 15-16 settembre 2022 • "3° corso di Diagnostica di laboratorio delle patologie neurologiche autoimmuni " Alghero- 06/08 ottobre 2022 • "Benessere psicologico tra operatori sanitari- Diventare consapevoli delle emozioni- Percorso Formativo di processo" AUO S. Luigi- 24/11-07/12/2022
Technology transfer achievements (patents, etc.):	NA

Paola Valentino, Medical Biotechnologist

Supervised PhD students:	NA
Honors, prizes, awards:	NA
Outreach activitiesNA	
• International collaborations:	NA
• Invited talks:	NA
• Science communication:	• 2/11/2022: "Energy efficiency vs. sample quality - Is there a fair trade-off? (ESBB webinar - 1h) • 26-28/10/2022: ECTRIMS 2022 38th Congress of ECTRIMS, 27th Annual Conference of RIMS, Amsterdam,The Netherlands (18 ECM, 22h) • 27/10/2022: Standard BioTools Webinar: Build Your Biobanking House on Solid Foot (ESBB webinar , 1h)

	• 25/10/2022: “From Bedside to Cryobench: Pre-Analytical factors and how to? (ESBB webinar -, 1h) • 13/10/2022-14/10/2022: Europe Biobank Week Roadshow 2022: Paediatric Biobanking and Minor Engagement (Ospedale Bambin Gesù,Roma) • 12/10/2022: Giornata Nazionale Biobanche BBMRI.it (Genova Symposia Organizzazione Congressi S.r.l) • 30/08/2022 : Management and Quality in Biobanking (ESBB webinar - 1h) • 28/07/2022: Broad consent or dynamic consent - an ongoing discussion (ESBB webinar -, 1h) • 22/07/2022: Webinar “Serum Glial Fibrillary Acidic Protein: A Biomarker of Disease Progression in Multiple Sclerosis” (Quanterix, 30 min) • 28/06/2022: ESBB Webinar: Human Biomonitoring: Biobanking & Exposure: Human samples and what they tell us about exposure to chemicals and health effects (ESBB webinar -, 1h) • 11/05/2022: Webinar: “Employing Serum NfL Measurements for Management of Multiple Sclerosis” (Genetic engineering and Biotechnology news, GEN, 1h) • 10-11/05/2022: Workshop: “Biobanking 101 Workshop” (ISBER, 6 h) • 03/05/2022-05/05/2022: Webinar: "Le infrastrutture di ricerca europee, BBMRI, EATRIS, ECRIN: opportunità e servizi offerti ai ricercatori (Istituto Superiore di Sanità, ISS, 7,5h) • 26/04/2022: Quality Management (ESBB webinar - 1h) • 08/04/2022: Serie Webinar: Accreditamento delle Biobanche in conformità alla UNI ISO 20387 “Biobanche e GDPR”. (Accredia, 3 ore) • 08/04/2022: Serie Webinar: Accreditamento delle Biobanche in conformità alla UNI ISO 20387 “Dati associati al materiale biologico. Sicurezza informatica”. (Accredia, 2 ore) • 18/03/2022: Serie Webinar: Accreditamento delle Biobanche in conformità alla UNI ISO 20387 “Focus sulla riferibilità metrologica”. (Accredia, 3 ore) • 28/01/2022: Serie Webinar: Accreditamento delle Biobanche in conformità alla UNI ISO 20387 “Focus sulle procedure di processo. (Accredia, 2 ore)
• Editorial duties:	NA
• others	Congress Posters:

	ECTRIMS 2022 • *sNFL applicability as additional monitoring tool in Natalizumab Extended Interval Dosing regimen for RRMS patients* (P. Valentino, S. Malucchi, S. Martire, C.I. Bava, M.A. Capobianco, A. Bertolotto) (EP1244) • *Profile of serum biomarkers in seronegative NMOSD* (S. Carta, A. Dinoto, M. Capobianco, P. Valentino, M. Lo Re, V. Chiodega, P. Branger, B. Audoin, J. Aboab, C. Papeix, N. Collongues, P. Kerschen, H. Zephir, A. Creange, B. Bourre, E.P Flanagan, V. Redenbaugh, G. Arrambide, J. Villacieros-Álvarez, A. Cobo-Calvo, S. Ferrari, R. Marignier, S. Mariotto) American Academy of Neurology (AAN) 2022 • *Applicability of sNFL in Multiple Sclerosis as Additional Measure to Monitor Treatments in Clinical Practice and Implications in NEDA-3 Evaluation* (S19.001) Engagement in BBMRI Working Groups: • "Metabolomics and Biobanks" • "Liquid biopsy" • "Implementation of UNI ISO 20387-pilot project" • "Population Biobanks" • "Biobancaggio/Biobanche di ricerca e interazione con il garante" (ELSI)
Organizational activities and responsibilities at NICO:	NA
Speakers invited:	NA
Other organizational activities:	Quality system management in CRESM biobank
Workshops, Schools or Conferences organized:	"*Ruolo dei Neurofilamenti nella diagnosi e monitoraggio dei pazienti con Sclerosi Multipla: il progetto pilota del CRESM*" (Webinar for North-West MS Network, 24/10/2022)
Technology transfer achievements (patents, etc.):	NA

Francesca Montarolo, PhD Biologist

Supervised PhD students:	NA
Honors, prizes, awards:	NA
Outreach activities	
• International collaborations:	NA
• Invited talks:	"*The role of MICROGLIA in Multiple Sclerosis: from MICRO to macro different point of view*", 28 settembre 2022, 5th Brainstorming Research Assembly for Young Neuroscientists (BRAYN), Roma. "*Sex-dependent hyperactive behaviour and altered microglial phenotype in NURR1 deficient mice*", 17 dicembre 2022, III Edizione More than Neurons, Torino.

• Science communication:	Webinar di approfondimento "Accreditamento delle Biobanche in conformità alla UNI ISO 20387- Focus sulla riferibilità metrologica", 18 marzo 2022, (online) (organizzatore ACCREDIA). Webinar di approfondimento "Accreditamento delle Biobanche in conformità alla UNI ISO 20387 - Biobanche e GDPR, 8 aprile 2022, (online) (organizzatore ACCREDIA). Webinar di approfondimento "Accreditamento delle Biobanche in conformità alla UNI ISO 20387 - Dati associati al materiale biologico – Sicurezza informatica, 8 aprile 2022, (online) (organizzatore ACCREDIA)
• Editorial duties:	Topic Editors at Frontiers in Cellular Neuroscience, Research topic: the cerebellar involvement in non-cerebellar pathologies
• others	NICO NeuroWebinar 04/02/2022 Congress participation with posters to the Annual Scientific Congress Italian MS society and its Foundation (FISM): - Air pollution and Multiple Sclerosis: role of particulate matter (PM) exposure and associated extracellular vesicle trafficking in neuroinflammation and demyelination. Roberta Parolisi, Francesca Montarolo, Luca Ferrari, Laura Dioni, Laura Cantone, Giulia Solazzo, Sabrina Rovelli, Andrea Cattaneo, Antonio Bertolotto, Annalisa Buffo, Valentina Bollati, Enrica Boda; - Activation of the MET receptor as therapeutic tool in MS: a new neuroprotective mechanism involving the glutamatergic system. Gallo Simona, Montarolo Francesca, Vitacolonna Annapia, Desole Claudia, Bertolotto Antonio, Vivien Denis, Comoglio Paolo, Crepaldi Tiziana
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

Serena Martire, Biostatistician

Supervised PhD students:	NA
Honors, prizes, awards:	NA
Outreach activities	
• International collaborations:	NA
• Invited talks:	Relatore nell'ambito del corso "Il trapianto autologo di cellule staminali Emolinfopoietiche nella sclerosi multipla (aHSCT)", FAD asincrona (organizzatore FISM)
• Science communication:	NA

• Editorial duties:	NA
• others	Congress Posters: ECTRIMS 2022 • *sNFL applicability as additional monitoring tool in Natalizumab Extended Interval Dosing regimen for RRMS patients* (P. Valentino, S. Malucchi, S. Martire, C.I. Bava, M.A. Capobianco, A. Bertolotto) (EP1244) American Academy of Neurology (AAN) 2022 • *Applicability of sNFL in Multiple Sclerosis as Additional Measure to Monitor Treatments in Clinical Practice and Implications in NEDA-3 Evaluation* (S19.001) Annual Scientific Congress Italian MS society and its Foundation (FISM) *Multiple Sclerosis disease activity and SARS-COV2 pandemic: a population based study from the Italian MS Registry* (Martire S, Lo Re M, Capobianco M on behalf of the Italian Multiple Sclerosis Register Centers Group, and the Scientific Committee of Italian MS Register)
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

Cecilia Bava, Molecular Biotechnologist

Supervised PhD students:	NA
Honors, prizes, awards:	NA
Outreach activities	
• International collaborations:	NA
• Invited talks:	Presentation in the Young Scientist session: “Comparison between live cell-based Fluorescence Activated Cell Sorting methodologies for diagnostic anti-MOG Ig detection”; 5° European NMOSD/MOGAD meeting (Lyon, 2-4/06/2022)
• Science communication:	"3° Corso di Diagnostica di Laboratorio delle malattie demielinizzanti" (Alghero, 7-8/10/2022; Organizzatore AINI)
• Editorial duties:	NA
• others	Posters - P. Valentino, et al. "sNFL applicability as additional monitoring tool in Natalizumab Extended Interval Dosing regimen for RRMS patients"(EP1244); Multiple Sclerosis JournalVolume 28, Issue 3_suppl: ECTRIMS 2022, p 1006-1007

	- Simona Malucchi, et al. "Applicability of sNFL in Multiple Sclerosis as Additional Measure to Monitor Treatments in Clinical Practice and Implications in NEDA-3 Evaluation" (S19.001) Neurology May 03, 2022
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

Lucia Giorgi, Cellular and Molecular Biologist

Supervised PhD students:	NA
Honors, prizes, awards:	NA
Outreach activities	
• International collaborations:	NA
• Invited talks:	NA
• Science communication:	"2° Corso di Diagnostica di Laboratorio delle malattie demielinizzanti" (Trento, 15-16/09/2022; Organizzatore AINI)
• 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

Antonio Bertolotto

Supervised PhD students:	NA
Honors, prizes, awards:	NA
Oureach activities	
• International collaborations:	NA
• Invited talks:[114]	COGNITIVITÀ E MALATTIE NEUROLOGICHE Torino, 12 ottobre 2022, Chairman SOGGETTI FRAGILI E COVID-19: GLI ANTICORPI MONOCLONALI PER PREVENIRE E PROTEGGERE, in Neurologia Torino, 18 ottobre 2022 Il percorso Sclerosi Multipla, Torino 2 novembre 2022 52° CONGRESSO SOCIETÀ ITALIANA DI NEUROLOGIA Milano, 3-6 Dicembre 2022, Chairman

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

• Science communication:[115]	
• Editorial duties:	Co-Editor-in-Chief "Neurology & Therapy" Academic Editor "Multiple Sclerosis International" Editorial Board: "Progress in Neuroscience" "Journal of Multiple Sclerosis" "Frontiers in Neurology – Multiple sclerosis and Neuroimmunology"
• others[116]	
Organizational activities and responsibilities at NICO:	NA
Speakers invited:	NA
Other organizational activities:[117]	Member of the Scientific Committee for Education of FISM (Federazione Italiana SM)
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	NA

ALL LAB MEMBERS

Activities:[118]	NA

[115] Public engagement
[116] Posters at meetings, participation in the board of scientific societies, referee for grant agencies
[117] No university appointments.
[118] 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 2022[119]

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 dedicated to both routine diagnostic and research activities, particularly focused on the identification and dissemination of biomarkers for diagnosis, prognosis and treatment efficacy in MS patients.

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 an early and proper treatment provides the best chance at slowing the progression of the disease. Identifying biomarkers able to anticipate the diagnosis, the prognosis and the response to treatment is crucial to give the patient an efficacious personalized therapy at an early stage of the disease.
Neurofilament light chain (NFL) are the most promising emerging biomarkers to monitor disease activity and progression, since they correlate with several clinical parameters including radiologic and clinical activity and treatment response. They are released upon axonal damage in the cerebrospinal fluid (CSF), and later in blood in lower concentrations. SIMOA technology, thanks to its ultra-sensitivity, has enabled the measurement of NFL in blood (serum and/or plasma), increasing the clinical applicability of this biomarker. However, routine serum NFL (sNFL) use is limited by the absence of immunological assays certified for diagnostics (CE-IVD), which are only available for CSF NFL quantification. The definition of reliable reference values to detect an increase of sNFL at individual level is also crucial for the dissemination of sNFL use in clinical practice. After purchasing the SR-X instrument (Quanterix) for sNFL quantification in 2018, we evaluated sNFL in a large real-life cross-sectional study. We defined specific decade-related cut-off values to discriminate high pathological from normal sNFL values, and identified MS patients showing pathological levels despite being clinically/radiologically stable during treatment. These results support the importance of sNFL in monitoring clinical and subclinical disease activity and response to treatment. On this basis, we applied sNFL dosing in the monitoring of MS patients treated with Natalizumab (NAT), that is one of the most effective treatment options. Unfortunately, NAT treatment is associated with the risk of developing progressive multifocal leukoencephalopathy (PML) in patients previously exposed to JC virus. The standard interval dosing (SID) protocol involves the administration of the drug once every 4 weeks, while an extended interval dosing (EID), every 6 weeks, has been recently proposed to minimize the risk. Studies showed that the efficacy of the NAT treatment, based on MRI and clinical evaluation, is maintained in the great majority of patients who switched to EID administration regimen. However, frequent MRI are expensive and not always applicable. Effective, accessible and easy-to-perform biological markers of disease activity are therefore needed.
The vast majority of biological research suffers from poor reproducibility of published data because of the lack of rigor in the collection of biological samples, the insufficient validation of the methods and limited sharing of data. This issue could be addressed by the creation of a structured Biobank able to collect, store and distribute data and samples obtained from MS patients to other researchers, following rigorous ethical and technical guidelines. The CRESM Biobank (BB-CRESM) is a non-profit organization mainly supported by FISM (Fondazione Italiana Sclerosi Multipla). It has been formally recognized by the AOU San Luigi in January 2020, and represents the first biobank recognized in the BBMRI network in Piedmont.

c. Objectives (1000 characters)

I) evaluating the correlation between sNFL and CSF NFL measured with SIMOA, and CSF NFL measured with a CE-IVD test (Uman Diagnostics), to add a further validation step towards routine sNFL use.

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II) assessing the applicability of sNFL as additional measure of treatment efficacy to monitor NAT-treated patients after shifting to EID approach. In particular, we aim to define if sNFL can be used as an alternative/additional measure to MRI for disease monitoring.
III) expanding the CRESM biobank through the collection of biological samples (serum, plasma, CSF, urine, cells from blood and CSF for DNA and RNA) and associated data of different types of MS or other neurological diseases and various controls, according to strict criteria.

d. Results (4000 characters)

I) CSF NFL levels measured with SIMOA and ELISA CE-IVD demonstrated a strong correlation, with ELISA CE-IVD underestimating CSF NFL values by 10% relative to SIMOA. sNFL (SIMOA) demonstrated a good correlation with both CSF NFL measured with SIMOA and ELISA CE-IVD. Finally, based on reference values for CSF NFL (ELISA CE-IVD, Uman Diagnostics) and for sNFL (previously published by our group), we obtained an 87% overall concordance between methods.
II) We performed a systematic evaluation and comparison of sNFL levels during SID and EID regimen in multiple longitudinal samples per patient. sNFL levels measured during SID and EID were comparable, without significant difference between groups, and intra-individual sNFL levels demonstrated overall stability during both SID and EID. According to our previously published reference values, sNFL levels were in the normal range in all samples, during both SID and EID.
Our results suggest that sNFL quantification can be used as an alternative/additional approach to MRI in managing individual patients.
III) Currently, 1630 participants are involved in BB-CRESM: they include about 1480 patients and 150 healthy individuals. In year 2022, 130 new participants were involved. BB-CRESM has been contacted and involved in several collaborations with public and private research institutions and pharma-companies. In 2022, more than 400 samples and associated data have been distributed. BB-CRESM implemented several operative documents to standardize and favor data and sample sharing, also outside of the European Union. It also worked to publicize its activity in scientific and social fields To guarantee the competence of its personnel, BB-CRESM has been involved in several working groups and training activities proposed by the main international network for biobanking BBMRI and ESBB.

e. Advancement in the field (1000 characters)

Results obtained support a new clinical application of sNFL to monitor NAT efficacy. In addition, we achieved a validation step to provide applicable, reliable, and robust sNFL results for routine use in clinical practice. This will improve the monitoring of disease activity and treatment efficacy, aiming at saving or better allocating NHS funds. Finally, BB-CRESM activity represents a crucial service to boost and accelerate research studies in the field of MS and neurological and autoimmune diseases.

f. Publications[120]

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

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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):

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):

The cause of MS is unknown, but it has a presumed autoimmune etiology. Accordingly, pregnancy acts as modulator of disease activity. 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. Thanks to the collaboration with Prof. 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 Turin (Italy), and with Prof. Stefania Bruno of the Department of Medical Sciences and Molecular Biotechnology Center, University of Turin, we collected placental tissues from both MS and healthy women, and we obtained a preliminary phenotypic characterization of placenta-derived extracellular vesicles (EV). Overall, our findings suggested a potential immunomodulatory role of placental EVs from women with MS and unveil some differences in their phenotype and functions compared to healthy women. Further studies on cell cultures and animal models, as well as the investigation of the EV molecular cargo, are required to unravel the mechanisms whereby placental EVs exert their beneficial effects on dysfunctional immune systems, and to direct future therapeutic interventions for patients with MS and other autoimmune diseases.
Aiming at addressing the need for reliable markers to monitor disease activity and treatment efficacy, thanks to the SR-X instrument (Quanterix) acquired in 2018 we have been focusing our efforts on implementing sNFL quantification in routine clinical practice. Besides NFL, glial fibrillary acidic protein (GFAP) is currently emerging as a promising biomarker of astrocytic damage and ongoing disease progression. High levels of GFAP, especially in combination with high NFL, have been suggested to be highly prognostic for future disability worsening, especially in patients with progressive forms of MS and with neuromyelitis optica spectrum disorder (NMOSD), for which definite prognostic and predictive biological markers are still lacking. However, many issues are to be assessed before GFAP can be applied in clinical practice, such as the absence of normative reference values and real-life large cohort studies.
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)

I) deepening our knowledge on the immunomodulatory potential of placental EVs. In particular, we plan to treat human blood cells with i) placental EVs from MS and healthy women and ii) serum EV from MS and healthy women who are not pregnant, in the 3rd trimester of pregnancy and in the puerperium, in the absence/presence of lymphocytes stimulation. Than we will evaluate and compare the proliferation of lymphocytes, the expression of phenotypic and activation markers by leucocytes and the release of cytokines

in the supernatant. In addition, we plan to explore the therapeutic effects of all the EV types 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.
II) continuing on the path of improving the sNFL measure in the routine clinical monitoring of MS patients. In particular we plan to: assess sNFL in a growing number of healthy individuals and patients with other neurological disorders; implement individual personalized cut-off values for MS patients; obtain a sNFL profile in different clinical contexts, as during the switch to other therapies and during pregnancy; implement the sNFL measurement service for the MS center network in the Northwest of Italy.
III) setting up GFAP dosing, taking advantage of the SIMOA technology and the expertise already acquired in sNFL testing. In particular, we aim to: define GFAP reference values in healthy controls; monitor disease progression of patients with primary progressive form of MS; identify and monitor relapsing-remitting MS patients transitioning to the secondary progressive form of MS.
IV) expanding CRESM biobank collection and distribution of biological samples/associated data for high quality research in the field of MS and other neurological disorders; creating a network of regional biobanks; implementing an appropriate process for the pediatric biobanking and new models for minor assent/consent; implementing an effective proper biobanking software for the management of samples and data: this is a crucial tool to enable the management of samples and data, to interface with the clinical management systems of the institution, according to privacy requirements and possibly to interface with external networks and platforms to facilitate the research and distribution of samples and data

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

I) Our studies on both placental and serum 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.
II and III) Optimizing the monitoring of disease activity and treatment efficacy will allow to save, or better allocate, enormous amounts of NHS funds.
III) The Biobank of the Clinical Neurobiology Laboratory will improve the reproducibility of data obtained by their users.

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 assessed by 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.

28/1/22 - Seminar

Giulia Ramazzotti, PhD

Department of Biomedical and Neuromotor Sciences, University of Bologna

Cell signaling pathways in autosomal-dominant leukodystrophy (ADLD): the intriguing role of the astrocytes

Autosomal-dominant leukodystrophy (ADLD) is an extremely rare fatal neurodegenerative disorder due to the overexpression of the nuclear lamina component, Lamin B1.The molecular mechanisms responsible for driving the onset and development of this pathology are not clear yet. Vacuolar demyelination seems to be one of the most significant histopathological observations of ADLD.
Considering the role of oligodendrocytes, astrocytes, and leukemia inhibitory factor (LIF)-activated signaling pathways in the myelination processes, we analyzed signaling alterations in different cell populations from patients with LMNB1 duplications and cellular models overexpressing Lamin B1 protein. Our results point out, for the first time, that astrocytes may be pivotal in the evolution of the disease. Indeed, cells from ADLD patients and astrocytes overexpressing LMNB1 show severe ultrastructural nuclear alterations, not present in oligodendrocytes overexpressing LMNB1. Moreover, the accumulation of Lamin B1 in astrocytes induces a reduction in LIF and in LIF-receptor levels with a consequential downregulation of downstream signaling pathways. Significantly, the toxic effects induced by Lamin B1 accumulation can be partially reversed, with differences between astrocytes and oligodendrocytes, highlighting that LMNB1 overexpression drastically affects astrocytic function reducing their fundamental support to oligodendrocytes in the myelination process. In addition, astrocytes overexpressing Lamin B1 show increased immunoreactivity for both GFAP and vimentin and also an increase in NF-kB phosphorylation and c-Fosactivation, suggesting the induction of astrocytes reactivity.
Therefore, Lamin B1 accumulation correlates with biochemical, metabolic, and morphologic remodeling, probably related to the induction of a reactive astrocytes phenotype that could be directly associated to ADLD pathological mechanisms.

Host: Annalisa Buffo

18/2/22 - Seminar

Alessandro Bertero, PhD

Armenise-Harvard Lab of Heart Engineering and Developmental Genomics
Molecular Biotechnology Center, University of Turin
Department of Molecular Biotechnology and Health Sciences

Functional dynamics of chromatin topology in human cardiogenesis and disease

Recent technological advancements in the field of chromatin biology have rewritten the textbook on nuclear organization. We now appreciate that the folding of chromatin in the three-dimensional space (i.e. its 3D “architecture”) is non-random, hierarchical, and highly complex. Nevertheless, functional changes in spatial genome organization during human development or disease remain poorly understood. We have investigated these dynamics in two models: (1) the differentiation of human pluripotent stem cells into cardiomyocytes (hPSC-CM); (2) hPSC-CM from patients with cardiac laminopathy, a genetic dilated cardiomyopathy with severe conduction disease due to mutations in the *LMNA*gene. We combined omics methods to probe nuclear structure (Hi-C), chromatin accessibility (ATAC-seq), and gene expression (RNA-seq), genetic perturbations by CRISPR/Cas9, and cardiac physiology assays. In this seminar I will summarize our published findings and present novel preliminary data that indicate the dynamic nature of genome organization during human development and disease, and show how these spatial relationships can regulate lineage-specific gene expression. Finally, I will describe the methods we are developing to probe the structure-function relationship of chromatin.

Host: Annalisa Buffo

Thursday 24/2/22 h. 2:00 pm - Internal Seminar, Group Buffo

Ben Vermaercke, Postdoctoral researcher

VIB-KU Leuven Center for Brain & Disease Research, Leuven Brain Institute, Belgium

Probing functional outputs of human transplanted neurons in mouse visual circuits

Host: Gabriela B. Gómez-González

4/3/22 h. 2:00 pm - Lecture

Angelo Forli, Ph.D.

Department of Bioengineering, University of California, Berkeley

Collective behavior and hippocampal activity in freely foraging bats

Social and collective behaviors are widespread across the animal kingdom, from ants to humans. Despite their prevalence and their role in shaping brain evolution, the neurobiological bases of collective behaviors remain largely unexplored. I will describe how I am attempting to address this challenge by (1) monitoring a group of Egyptian Fruit bats – highly social mammals – collectively foraging in a

large laboratory room and (2) by recording the activity of single neurons in their hippocampus, a fundamental brain region for navigating physical and social space.
Host: Serena Bovetti

18/3/22 h. 4:00 pm - Seminar
Dilek Colak, Ph.D.
Assistant Professor of Neuroscience, Feil Family Brain and Mind Institute, Center for Neurogenetics
Assistant Professor of Pediatrics, Gale and Ira Drukier Institute for Children's Health
Weill Medical College, Cornell University, New York
Astrocyte dysfunction in ASD
The cellular mechanisms of autism spectrum disorder (ASD) are poorly understood. Cumulative evidence suggests that abnormal synapse function underlies many features of this disease. Astrocytes regulate several key neuronal processes, including the formation of synapses and the modulation of synaptic plasticity. Astrocyte abnormalities have also been identified in the postmortem brain tissue of ASD individuals. To address this, we combined stem cell culturing with transplantation techniques and demonstrated that astrocytes derived from ASD iPSCs are sufficient to induce repetitive behavior as well as cognitive deficit in experimental animals, suggesting a previously unrecognized primary role for astrocytes in ASD.
Host: Annalisa Buffo

1/4/22 h. 4:00 pm - Lecture
Shi-Bin Li, Stanford University
Interrogation of sleep disorders associated with aging and stress
We spend approximately a third of our lives asleep. High-quality sleep is essential to maintain our physical and mental health. A good night sleep not only restores our physical and mental strength efficiently, but also helps us to maintain mental health and strengthen immunity. However, sleep is subjected to various challenges including aging and stress, across the lifespan. Sleep quality declines with age. The elderly usually experience low-quality sleep including difficulty to fall asleep, reduction in slow-wave/deep sleep, early waking up, and prominently sleep fragmentation which heavily impairs the ability of sleep in restoring physical and mental strength. Stress not only prevents us from a good night slumber, but may also make us more vulnerable to pathogen exposure. Around these topics, Dr. Li and colleagues accumulated some evidence showing a mechanistic underpinning of sleep instability with age, and a hypothalamic circuitry underlying stress-induced hyperarousal/insomnia and peripheral immunosuppression.
Host: Ilaria Bertocchi

13/5/22 h. 2:00 pm - Webinar
Stefano Zucca (Group Peretto - Bonfanti)
Breaking the stigma on Academic Mental Health
In the past 10 years there has been an increased attention on mental health and wellbeing among people working in academia. A wide range of scientific papers, local and global surveys together with articles and commentaries highlight a worrying situation about mental health issues among PhD students and early career researchers. Long working hours, competition, job insecurity and toxic working environments are causing extremely high stress levels in academia. Recent evidence shows that nearly 20% of PhD students suffer from diagnosable anxiety or depression. In this talk, I will provide an overview of what we know about mental health and wellbeing in academia, focusing on common stressors among researchers. I will cover main factors contributing to high stress levels in academic working environments and I will propose possible solutions to manage researchers' stress and improve their wellbeing. Discussing and raising awareness about mental health in academia is a fundamental step to fight stigma and help people seeking help, if and when they need.

10/6/22 h. 2:00 pm - Seminar
Elia Ranzato, Università del Piemonte Orientale
Honey and tissue regeneration: an unusual Ca2+ affair
Honey and other honeybee products may represent a very attractive compounds for wound repair. We will understand how and why.

Thursday 23/6/22 h. 2:00 pm - Seminar
Letizia Mariotti, University of Padova
A genetically identified class of premotor neurons coding for head movements in the mouse superior colliculus
The success of the simple daily routine of grasping a cup of coffee before going to work requires the ability to use sensory information to evaluate the relative position of the self and the target in space and finally execute the intended movement.
A crucial sensory-motor hub in the brain that guides similar goal-oriented head movements is the superior colliculus (SC). However, it is unknown how neuronal circuits in the SC trigger head movements in different directions, what are the classes of neurons involved, and what inputs from the cortex are required in the process.
To answer these questions, we genetically dissect the murine SC, identifying a functionally and genetically homogenous subclass of glutamatergic neurons expressing the transcription factorPitx2. We demonstrate that the optogenetic stimulation of $Pitx2^{ON}$ neurons drives three-dimensional head displacements characterised by stepwise, saccade-like kinematics. Furthermore, during naturalistic foraging behaviour, the activity of $Pitx2^{ON}$ neurons precedes the onset of spatially-tuned head movements. Finally, we reveal that $Pitx2^{ON}$ neurons are clustered in orderly array of anatomical modules that tile the entire motor layer of the SC. Such a modular organization gives origin to a discrete and discontinuous representation of the motor space, with each $Pitx2^{ON}$ module subtending a defined portion of the animal's egocentric space. Overall, these data support the view of the superior colliculus as a selectively addressable and modularly organised spatial-motor register.
Host: Stefano Zucca

18/7/22 h. 2:00 pm - Seminar
Pritz Christian Oliver, Hebrew University of Jerusalem, Israel
Principles for coding associative memories in a compact neural network
Host: Ferdinando Di Cunto

22/7/22 h. 2:00 pm - Seminar
Marco Tripodi, MRC Laboratory of Molecular Biology, Cambridge
The space of actions - Neural circuits for transforming spatial representations into actions
Host: Alessandro Vercelli

Tuesday 26/7/22 h. 10:00 am - Seminar
Nir Giladi, Tel Aviv University, Israel
Genetic aspects of Parkinson's disease, a lesson learnt from Ashkenazi Jews
Host: Alessandro Vercelli

Thursday 6/10/22 h. 12:00 am - Seminar
Ludovico Silvestri, LENS (European Laboratory for Non-Linear Spectroscopy) and University of Florence, Italy
Adaptive and smart light-sheet microscopy: a dimensional leap in neuroscience and biology
Traditionally, histological analysis of biological samples involved tissue slicing followed by pure 2D reconstructions. This sampling strategy wastes a lot of precious information about the molecular and cellular architecture of the specimen and can introduce biases due the choice of slice and of the cut orientation. In this scenario, light-sheet fluorescence microscopy (LSFM), coupled with chemical clearing of tissue, surged as a potential game changer allowing full volumetric reconstruction of entire organs with sub-cellular resolution. However, despite the great promise hold by this method, its routine use is still often limited to the production of a couple of fancy 3D renderings without any real biological insight. In this talk, I will analyze the optical and computational limitations of state-of-the-art LSFM, and discuss our recent advances to achieve scalable, robust, and quantitative analysis of macroscopic tissue samples. Finally, I will describe some applications of this "adaptive and smart" microscopy, from the dissection of brain-wide circuits involved in fear memory to the architectural analysis of the Broca's area in the human brain, to 3D analysis of surgical specimens which could prospectively improve diagnostic accuracy.
Host: Annalisa Buffo/Roberta Parolisi

14/10/22 h. 2:00 pm - Seminar
Nicoletta Filigheddu, DIMET - Dipartimento di Medicina Traslazionale, Università del Piemonte Orientale, Novara, Italy
A new role for vitamin D binding protein
Vitamin D binding protein (VDBP), as its name suggests, is the primary carrier of vitamin D in the blood. Nevertheless, it has many physiological functions, including a role in the scavenging system of the intracellular globular actin released after tissue damage. Curiously, high levels of VDBP have been reported in biological fluids of patients affected by pathologies associated with muscle wasting and weakness, suggesting that VDBP could contribute to this phenotype. In this talk, I will present an overview of the effects of VDBP on the skeletal muscle, providing evidence that VDBP acts as a hormone per se with pro-atrophic activities that depend on the perturbation of the intracellular actin dynamic and include mitochondrial dysfunction and neuromuscular junction dismantling.
Host: Serena Stanga

Tuesday 25/10/22 h. 2:00 pm - Seminar
Stefano Suzzi, Weizmann Institute of Science, Israel
Two stories, one message: loss of brain-immune homeostasis threatens brain function
Alzheimer's disease (AD) is anenigmatic neurodegenerative disease, since brain pathology is not sufficient to explain functional loss. The elucidation of the anatomical and functional relationships between the brain and the immune system has revolutionized the concept of "immune privilege". It is now clear that factors affecting the immunological milieu outside the brain or at its borders also shape the brain's fate. In one project, we found that high-fat obesogenic diet accelerated disease manifestations in a mouse model of AD (5xFAD). We found that the early onset was linked to systemic $CD4^+$ T-cell deregulation reminiscent of immune aging, and to increased circulating levels of free *N*-acetylneuraminic acid (NANA).
We demonstrated that NANA could recapitulate diet-induced immune perturbations and accelerate cognitive deterioration when administered to regularly-fed 5xFAD mice.
In a second project, we focused on the choroid plexus (CP) as a key immune gatekeeper of the brain. We show that the CP epithelium expresses the neuronal-selective cholesterol 24-hydroxylase CYP46A1, and that its product 24-hydroxycholesterol (24-OH) can locally suppress immune-related signatures previously associated with cognitive impairment. We found that CYP46A1 expression by the CP is reduced in 5xFAD mice, and that boosting its expression is neuroprotective.
In summary, we propose that therapeutic approaches targeting systemic immunity or the brain's borders are potentially disease-modifying strategies regardless of primary etiology.
Host: Alessandro Vercelli

28/10/22 h. 2:00 pm - Seminar
Laura Gioiosa, Department of Medicine and Surgery, University of Parma, Italy
THE 4TH S: THE IMPORTANCE OF SEX IN THE LAB
Scientific evidence indicates that sex and gender affect health and disease susceptibility in both animal models and humans. Despite several official calls recommend the inclusion of both sexes and/or the report of sex as an experimental variable, animal research continues to preferentially use males over females. As a result, female subjects are still under-represented in basic and preclinical research. This is unfortunate considering that biological sex differences have been observed at multiple levels and in different phenotypes, from behavior to physiology, to susceptibility to stressors and diseases. This talk reviews several studies on sex differences in different fields of neuroscience, from social and emotional behavior to response to stress, with particular emphasis on sex-dependent

effect of common experimental procedures. I will highlight: (a) the importance of sex as a biological variable when designing an experiment; (b) how during development and later in life social and environmental factors, and in particular common laboratory and experimental procedures, can differentially affect male and female behavioral and physiological responses; (c) the importance to unravel factors contributing in sexual differences that confer differential vulnerability to disease; and finally (d) how, in the perspective of good science, not only is it particularly important to carefully consider species/strain/genotype but also the sex of experimental animals.
Host: Group Gotti

Thursday 10/11/22 h. 2:00 pm - Seminar
Stas Engel, Ben-Gurion University of the Negev
Targeting pathogenic β6/β7-loop epitope of misfolded SOD1 - a potential therapeutic strategy for ALS
The current strategy to mitigate the toxicity of misfolded SOD1 in familial ALS is by blocking SOD1 expression in the CNS. Being indiscriminative toward misfolded and intact SOD1 proteins, such treatment, however, entails a risk of depriving the CNS cells of their essential antioxidant potential. We developed scFv-SE21 intrabody to block the β6/β7 loop epitope exposed exclusively in misfolded SOD1, as an alternative approach to neutralize misfolded SOD1 species and spare unaffected SOD1 proteins. ScFv-SE21 expression in the CNS of hSOD1G37R mice rescued spinal motoneurons, reduced the accumulation of misfolded SOD1, decreased gliosis, and thus delayed disease onset and extended survival by 90 days. Our results provide evidence that the exposure of the β6/β7 loop epitope is part of the pathogenic mechanism of misfolded SOD1, and raise the possibility that its blocking may constitute a novel therapeutic approach for ALS, with a reduced risk of collateral oxidative damage to the CNS.
Host: Marina Boido

Monday 14/11/22 h. 2:30 pm - Seminar
Bianca Silva, CNR, Humanitas
Brain circuits for fear attenuation
How are consolidated memories modified on the basis of experience? In the lab we aim to unravel the neural mechanisms at the basis of memory update. Understanding this biological process allows us to decipher how new information is constantly incorporated into existing memory, how a newly formed memory is integrated into previous knowledge and how the fine balance between memory stability and memory flexibility is maintained.
By using fear memory extinction as a model of memory update, we combine neuronal circuit mapping, fiber photometry, chemogenetic and closed-loop optogenetic manipulations in mice, and showed that the extinction of remote (30-day old) fear memories depends on thalamic nucleus reuniens (NRe) inputs to the basolateral amygdala (BLA). We find that remote, but not recent (1-day old), fear extinction activates NRe to BLA inputs, which become potentiated upon fear reduction. Both monosynaptic NRe to BLA, and total NRe activity increase shortly before freezing cessation, suggesting that the NRe registers and transmits safety signals to the BLA.
Accordingly, pan-NRe and pathway-specific NRe to BLA inhibition impairs, while their activation facilitates fear extinction.
These findings identify the NRe as a crucial BLA regulator for extinction, and provide the first functional description of the circuits underlying the experience-based modification of consolidated fear memories.
Host: Stefano Zucca

Friday 25/11/22 h. 3:00 pm - Seminar
Marco Terenzio, OIST, Okinawa
Dynein Roadblock 1 mediates axonal transport and degradation of FMRP1 in sensory neurons
Cytoplasmic dynein mediates axonal retrograde transport, thus playing a crucial role in conveying peripheral signals in neurons. Roadblock 1 (DYNLRB1) is one of dynein's three light chains and was shown to control lysosomal axonal transport and mediate survival signaling in sensory neurons. To identify the nature of these signals we used a proximity-dependent biotinylation approach coupled with mass spectrometry. Among other candidates, we identified the Fragile X mental retardation protein (FMRP), an RNA-binding protein with implications in neurological diseases.
We found that FMRP1 associates with DYNLRB1 in axons and is retrogradely transported. Combined shRNA-mediated silencing of DYNLRB1 with pharmacological treatments targeting the proteolytic machinery, we showed that DYNLRB1 knockdown reduces FMRP transport and degradation, causing axonal accumulation of FMRP protein granules. Increase colocalization with lysosomes was also detected, suggesting a novel degradation route for FMRP, which is generally believed to be cleared primarily by ubiquitination, in sensory neurons. The observed increase in FMRP granules likely causes the sequestration of FMRP-associated mRNAs, including MAP1B mRNA. Indeed, we found that DYNLRB1 knockdown in sensory neurons reduces MAP1B translation. Thus, our findings suggest that DYNLRB1-FMRP interaction controls FMRP function through the promotion of its transport and targeted degradation. This mechanism could have a prominent role in the etiology of neurodegenerative diseases.
Host: Letizia Marvaldi

Wednesday 7/12/22 h. 12:00 am - Seminar
Maria Concetta Miniaci, Professor of Physiology
University of Naples Federico II, Department of Pharmacy
Role of Locus Coeruleus-Norepinephrine System in Fear Conditioning
Norepinephrine (NE) is a neuromodulator involved in a broad variety of brain processes, including attention, arousal, decision making, and memory. The cerebellar cortex receives a widespread noradrenergic projection from the locus coeruleus (LC) which is consistent with the evidence that the NE system is involved in the modulation of cerebellar functions including motor learning. By using optogenetic and chemogenic approaches in mice, Maria Concetta Miniacihas demonstrated *in vivo* that the LC projections to the cerebellum plays a critical role in fear memory formation. In addition, she showed that, following fear conditioning, the conditioned stimulus elicits the release of NE in the cerebellum.
According to the electrophysiological data, NE modulates one of the main excitatory synapses in the cerebellum, i.e. the parallel fiber-Purkinje cell (PF-PC) synapse, by acting on α- and β -ARs. In particular, the activation of α-ARs produces synaptic depression between PFs and PCs whereas β2-AR activation facilitates the PF-PC synaptic potentiation. This double mechanism of regulation of PF-PC synaptic transmission by NE may serve to decrease the background activity of PCs and enhance the excitatory signals arriving at PCs via PF. In such a way, the NE release can refine the signals arriving at cerebellum at particular arousal states or during learning.
Host: Annalisa Buffo/Filippo Tempia

Thursday 15/12/22 h. 10:00 am - Seminar

Dr. Carmen Falcone - SISSA, Trieste, Italy

Cortical astrocytes across mammalian evolution: the special features of interlaminar astrocytes and varicose-projection astrocytes

Cortical astrocyes show an impressive heterogeneity across mammals. While protoplasmic and fibrous astrocytes have been observed in all mammals, there are two types of astrocytes with special features in primates: the interlaminar astrocytes (ILAs) and the varicose-projection astrocytes (VP-As). The interlaminar astrocytes (ILAs) are a subset of Glial fibrillary acidic protein $(GFAP)^{+}$ astrocytes with singular morphological traits: they can be identified in the cerebral cortex by having a cell body in the most marginal layer of the cerebral cortex (layer I), very close to the pia, and long, interlaminar processes running into deeper cortical layers, reaching layer V in humans. We compared ILA morphology, density and molecular markers across mammalian evolution and development,and found they have special features in primates. VP-As, instead are a special type of astrocyte observed in hominoid species only. VP-As are usually visible in the deeper layers of the cortex and show peculiar varicosities (beads) along their longest processes. We analyzed the presence and appearance of VP-As across multiple species of primates, with a special focus on apes and humans, we described their distribution and their expression of specific astrocyte markers across species. In this talk, I will show data resulted from these two studies, and will discuss potential relevance for future functional studies of astrocytes in development and evolution.

Host: Valentina Cerrato

PROGRESS REPORT

14/1/22 - Progress report
Brigitta Bonaldo (Group Panzica)
Effects of perinatal exposure to bisphenol A or S in EAE model of multiple sclerosis.

21/1/22 - Progress report
Roberta Schellino (Group Vercelli)
Long-term transplantation and enriched environment favor human striatal progenitor maturation and functional recovery in a rat model of Huntington's Disease

4/2/22 - Progress report
Francesca Montarolo (Group Capobianco)
Age- and sex-dependent behavioral phenotypes in NURR1 deficient mice

11/2/22 - Progress report
Maryam Khastkhodaei Ardakani (Group Buffo)
Rescuing neural cell survival and maturation in a microcephaly 17 (MCPH17) model: effects of postnatal and in utero N-acetyl cysteine treatments

25/2/22 h. 2:00 pm - Progress report
Ilaria Bertocchi (Group Eva)
Role of perineuronal nets in fragile X syndrome
11/3/22 h. 2:00 pm - Progress report
Gabriela Berenice Gomez Gonzalez (Group Buffo)
Assessing the functional integration of hESC-derived striatal grafts in a rat model of HD by calcium photometry

14/4/22 h. 2:00 pm - Progress report
Gianmarco Pallavicini (Group Di Cunto)
Human and mice neurodevelopment, how models change findings

22/4/22 h. 2:00 pm - Progress report
Giovanna Menduti (Group Vercelli)
Moxifloxacin rescues Spinal Muscular Atrophy phenotypes in both animal model and patient-derived cells

6/5/22 h. 2:00 pm - Progress report
Martina Lorenzati (Group Buffo)
Human IPSCs-derived oligodendrocytes and astrocytes as the first Autosomal Dominant Leukodystrophy-relevant cellular models

20/5/22 h. 2:00 pm - Progress report
Sara Bonzano (Group Bonfanti-Peretto)
A Pilot Investigation of Nr2f1 expression and functions during Experience-dependent Neuroplasticity in the Adult Mouse Dentate Gyrus

17/6/22 h. 2:00 pm - Progress report
Stefano Zucca (Group Bonfanti-Peretto)
Whole brain representation of imprinted cues

1/7/22 h. 2:00 pm - Progress report
Anna Caretto (Group Vercelli)
Hypothesis of glycinergic system alterations in Spinal Muscular Atrophy

15/7/22 h. 2:00 pm - Progress report
Marco Fogli (Group Bonfanti-Peretto)
Continuous turnover of astrocytes-derived niches supports long-term neurogenesis in the lesioned striatal parenchima

9/9/22 h. 2:00 pm - Progress report
Giorgia Iegiani (Group Di Cunto)
CITK loss leads to DNA damage accumulation impairing homologous recombination by BRCA1 mislocalization

23/9/22 h. 2:00 pm - Progress report
Valeria Vasciaveo (Group Tamagno)
Sleep fragmentation affects glymphatic system function through the different expression of AQP4 in wild type and 5xFAD mouse model

7/10/22 h. 2:00 pm - Progress report
Daniela Maria Rasà (Group Vercelli)
An in vitro study to preliminarily assess the stressor effects on Amyotrophic Lateral Sclerosis onset and progression

21/10/22 h. 2:00 pm - Progress report
Eleonora Dallorto (Group Bonfanti-Peretto)
A study on the effects of NR2F1 haploinsufficiency in the postnatal hippocampus

Thursday 3/11/22 h. 2:00 pm - Progress report
Ilaria Ghia (Group Bonfanti-Peretto)
A multidisciplinary study on the olfactory dopaminergic population and its role in processing sexual odors

Friday 2/12/22 h. 2:00 pm - Progress report
Martino Bonato (Group Buffo)
Citron-kinase regulates oligodendrocyte differentiation via cell autonomous and non-cell autonomous mechanisms.

Friday 16/12/22 h. 2:00 pm - Progress report
Gianna Pavarino (Group Vercelli)
Health benefits of living in close proximity to greenery: preliminary results on depression

Wednesday 21/12/22 h. 2:00 pm - Progress report
Marta Ribodino (Group Buffo)
Neuroanatomical and functional integration of human striatal neurons into a rodent model of Huntington Disease