Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Annual Report 2023

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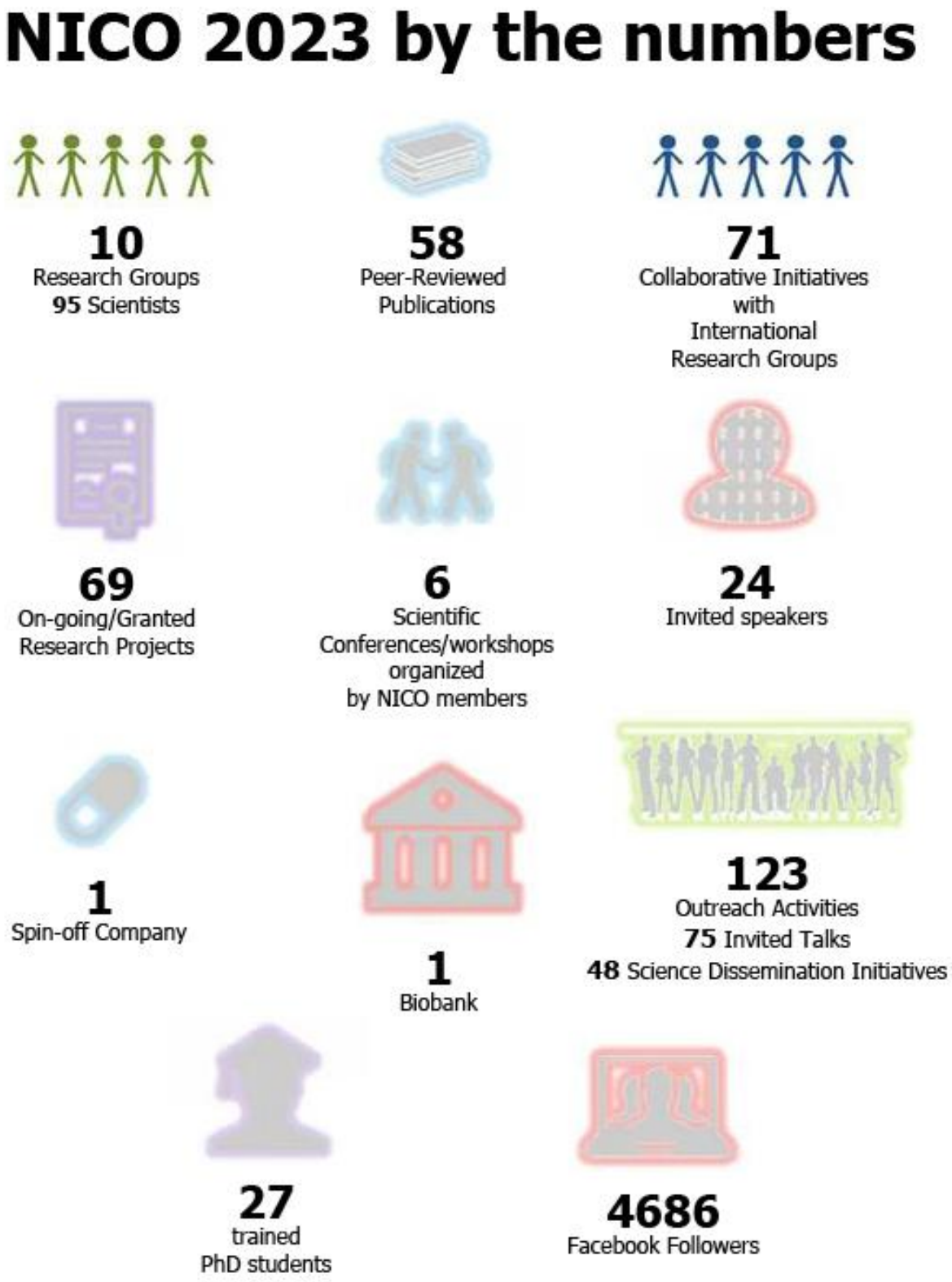
NICO 2023 by the numbers
10
Research Groups
95 Scientists
58
Peer-Reviewed
Publications
71
Collaborative Initiatives
with
International
Research Groups
69
On-going/Granted
Research Projects
6
Scientific
Conferences/workshops
organized
by NICO members
24
Invited speakers
1
Spin-off Company
1
Biobank
123
Outreach Activities
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48 Science Dissemination Initiatives
27
trained
PhD students
4686
Facebook Followers

BRIEF HISTORICAL NOTES

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

After decades in which the Foundation has funded scientific research projects and purchase of scientific equipment, 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 27 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's and master's degrees. In particular, NICO researchers are directly involved in the organization of the master's degree in biotechnology for Neuroscience (of prof. Di Cunto and Boido are respectively president and vice president).

Currently, NICO laboratories host around 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.

In particular, NICO is a key element in the project of Excellence of the Department of Neuroscience, since it will be fundamental in a) creating a C. Elegans lab for modelling neurodegenerative diseases, b) creating an IPSC and organoid platform to study neural diseases and new drugs and finally c) directing part of the research to space, in the frame of the regional and industrial plans for the "Cittadella dello Spazio".
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

In addition to the one with UNITO, NICO has signed several formal agreements to host students of the Universities of Camerino, Insubria, Palermo, Parma, Siena, Trieste and Verona.
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, (virtual) 2021 and 2024 editions were organized with the administrative help of the Ottolenghi Foundation.
In 2022, Prof. Boido and Dr. Stanga organised for the second time a biannual international workshop on motoneuron diseases which was attended by 115 people; they are preparing a new edition for the end 2024.
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 to December 2023 the NICO scientific director, A. Vercelli was acting as president of the Italian Society for Neuroscience. The National meeting of the Society in 2023 (755 attendees) has been held in Torino and Prof. Di Cunto has served 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. In 2023 a visit to several Universities in Japan (University of Tokyo, University of Osaka and Riken Institute at Kyoto) and to Brazil (Sao Paulo, Minas Gerais, Brasilia, Belo

Horizonte and Rio de Janeiro) have been performed to explore the possibilities of joint projects and collaborations. These will be finalized in 2024. In the meantime, A. Vercelli has met the governance of the Medical University of Wenzhou, China to discuss future 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).
Researchers at NICO participate to the PNRR projects in the frame of the European "Recovery Europe" D34H (digital and biological twin of the patient), Innova, Nodes and TO Move.

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." Prof. Boido represents the NICO in the network.

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 on 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, a **consultant for fund raising**, Dr. Alessandra Gerbo, 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**: 7 full professors, 14 associate professors, 8 university research assistants, 3 technicians, 24 post-docs/bursaries and 27 doctoral students;
- **Hospital staff**: 1 manager biologist, 2 specialists in Clinical Biochemistry, 2 post-doc fellows, 1 laboratory technician.

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

During 2023, the significant increase in the number of researchers at different levels of their career forced to convert a meeting room in the office of postdocs, and to create a room for technicians and another for research assistants.

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 UltraMicroscope 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 2022-23

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 has been delivered in December 2023. Thanks to the funding of the project of excellence, for the period 2023-27, the Department of Neuroscience is acquiring new instrumentation for the NICO facilities (for instance the Zeiss Apotome).
We are also planning to acquire a super resolution confocal microscope and systems to measure neural electrical activity in vivo and in vitro in 2024.

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. He has been replaced by Dr. A. Di Sapio, chief of the Hospital Neurology Unit.
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 December, our community was deeply struck by the sudden loss of Prof. Carola Eva, PI of the group Neuropsychopharmacology, professor of Pharmacology UNITO and one of the founders of NICO. While we mourn her death, we will remember her in future official events as a mentor and an inspiring colleague.

Upcoming projects on instrumentation, personnel and facilities
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 2023 more than 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€, and for the PNRR project INNOVA (Ministry of Health) and collaborate to the project TO Move. Prof. Boido and Calì participate in the PNRR project NODES 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 8 M€, in the field of Basic Neuroscience: the project was written by A. Vercelli.
The current agreement between UNITO and FCO foresees a contribution from UNITO to FCO of the 50% of the running costs of NICO from UNITO (205.000 € in 2023). 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. Recently, 12 projects from NICO researchers were financed by the PRIN plan of the Ministru of Research.
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 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. In the frame of the agreement with the University of Torino, a contribution to running costs (50%, 205.000 € in 2023) We foresee further increases due to the involvement of NICO in PNRR grants as a private entity.

Fund raising

In mid-2023, a collaboration was also started with a strategic fundraising and philanthropy consultant (dr. Alessandra Gerbo), in order to identify new projects and propose them to potential new partners, simultaneously starting a reflection on the possibilities of raising funds from individuals.

PLANS FOR INTERNATIONALIZATION

Following the recruitment of Dr. Ciola, the scientific director has started an important plan for internationalization. In his capacity of deputy rector for Biomedical Research, he has participated to three institutional academic journeys in different countries, Israel (2022 and 2023), Japan and Brasil (2023). In the frame of these visits, the NICO has been presented to the universities and organisations met during the visit. Many exchanges of researchers, both for seminars and projects, have been planned. In addition, discussions have been undertaken to create a world network of Neuroscience institutes of excellence (for instance, Instituto Cajal in Madrid, IDOR in Rio de Janeiro, University of Osaka, Kyoto University and Riken Institute in Tokyo). Moreover, thanks to Prof. Vercelli's initiative, an agreement has been defined to join the Network Medicine Institute and Global Alliance representing 33 leading universities and institutions around the world committed to improving global health and advancing the field of Network Medicine. This will allow to participate participating to consortia across the Atlantic to prepare applications for funding on both sides of it. The signature ceremony will take place in Boston at the end of March.

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 2023

- Brain in the wood, public talk at the National Museum of the Mountains (26 January);
- Unistem Day (10 March);
- Olympic Games of Neuroscience (February 10 - March 18);
- Brain Awareness Week (March 14-20)
- Science Weeks (March to May);
- Participation to the Turin Book Fair (September) in the UNITO stand;
- Researcher's Night UNIGHT (September 30)
- Ricercatori alla spina (Scientific events in pubs)

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). 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.

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
27 seminars were held on Friday afternoons. For invited speakers, see the attached list. In addition, a program of internal progress reports was organised.

NEW CHALLENGES
The perceived need for change must be contextualized within a scenario of profound transformation that the Foundation and NICO are going through, produced by the convergence of three main instances. The first is represented by the prospect, in 2/3 years, of transferring the Institute and its laboratories to the new university center which is being built in the area of the so-called "Ex Scalo Vallino" in Via Nizza, with the consequent disposal of the structure in Orbassano, which currently has onerous management costs. From this perspective, further resources could therefore become available to fuel the organisation's mission. Secondly, the Reform of the Third Sector, which in some way forces the Foundation to deal with a profoundly evolving scenario and to have to evaluate possible changes to best seize the opportunities that will arise. Thirdly, the need to adapt the operational and management structure of the Foundation to new and more complex needs triggered, for example, by digitalisation, by the increase in economic resources to manage and by competition from other non-profit entities on the market for donations from private.
In light of the profound changes underway and with a view to enhancing the potential demonstrated by NICO, the Foundation therefore feels the need to return to focusing attention on itself, on the organization and on the processes that characterize it, in order to adapt to the new context and to be able to grasp the potential that flows through it in the design of its medium-long term development strategy.

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2023

Laboratory name: **Adult neurogenesis**

1. LABORATORY DESCRIPTION – PERSONNEL:

Principal Investigator

Principal Investigator 1

LUCA BONFANTI
Degree: DVM, PhD
Birthdate: 19/05/1962
Nationality: Italian
Gender: M
Phone: 00 39 011 6706606
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Principal Investigator 2

PAOLO PERETTO
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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

3. SERENA BOVETTI
Degree: PhD
Birthdate: 13/09/1977
Nationality: Italian
Gender: F
Phone: 00 39 011 6706613
Email: serena.bovetti@unito.it
Position: Associate professor
Role & Expertise: Lead researcher on the study of neural network involved in sexual imprinting

4. SARA BONZANO
Degree: PhD Birthdate: 22/03/1987
Nationality: Italian Gender: F
Phone: 00 39 011 6706632
Email: sara.bonzano@unito.it
Position: Postdoc - Assistant professor (RTD-A) since july 2023
Role & Expertise: Cellular and molecular analyses of AN in the hippocampus; morphometric assessment on mitochondria *ex vivo*

5. STEFANO ZUCCA
Degree: PhD Birthdate: 29/05/1988
Nationality: Italian Gender: M
Phone: 00 39 011 6706632
Email: stefano.zucca@unito.it
Position: Marie-Curie Fellow
Role & Expertise: two-photon and lightsheet microscopy, electrophysiology

6. ALESSANDRA STELLA
Degree: PhD Birthdate: 29/05/1988
Nationality: Italian Gender: F
Phone: 00 39 011 6706632
Email: alessandra.stella@unito.it
Position: Postdoc
Role & Expertise: mathematical and statistical analysis

7. MARCO GHIBAUDI
Degree: Biological Sciences Birthdate: 29/05/1992
Nationality: Italian Gender: M
Phone: 00 39 011 6706632
Email: marco.ghibaudi@unito.it
Position: Postdoc (Assegno di ricerca)
Role & Expertise: Cellular and molecular analyses of immature neurons in mammals

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

10. 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)
Role & Expertise: Histology, confocal microscopy, morphometric analysis, mouse models

11. 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	Funding Program/Agency	Role of the unit	Overall Amount Funded	Managed by FCO/UNITO
May 2022 – November 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 Euro	UNITO
October 2022 – November 2024	Neuroni 'immaturi' come riserva di	Luca Bonfanti	Fondazione CRT (bandi ordinari)	Coordinator	27,000 Euro	FCO

	cellule indifferenziate 'dormienti' nella corteccia cerebrale umana					
October 2023 - September 2025	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	PRIN2022	Coordinator	65,000 Euro	UNITO
November 2023-October 2025	Nr2f1-dependent regulation of Mitochondrial Function in Neural Development and Disease	Silvia De Marchis	PRIN 2022	Coordinator	71,466	UNITO
December 2023-November 2025	The role of miR-211 in neuronal aging: From Disease Mechanisms to Therapy	Silvia De Marchis	PNRR-PRIN 2022	PI	79,685	UNITO
November 2020 – October 2024	Sounds and pheromones: neural networks merging olfactory and acoustic cues	Serena Bovetti	Human Frontier Science Program	Coordinator	350.000 $/3 years	UNITO

	in sexual imprinting					
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 acoustic cues in mouse courtship communication	Serena Bovetti/Stefano Zucca	H2020 Marie Sklodowska Curie Action Individual Fellowship	Coordinator/ Recipient	39.000 euro	UNITO
January 2023-Dec 2023		Serena Bovetti	Grant for internazionalization	Coordinator	12.500 euro	UNITO
2023-2025	Discovering the Effectors of Lifestyle-driven Memory enhancement via Inflammation	Serena Bovetti	PRIN-PNRR 2022	PI	81.000 euro	UNITO
2022-2024	Project support Grant	Stefano Zucca	British Society of Neuroendocrinology.	Recipient	6.700 euro	UNITO

3. SCIENTIFIC ACTIVITIES IN 2023

Luca Bonfanti, Associate professor (PI)

Supervised PhD students:	Alessia Pattaro (second year) Marco Ghibaudi (Assegno di ricerca)
Honors, prizes, awards:	n.a.
Outreach activities	
• International collaborations:	Sebastien Couillard-Despres, University of Salzburg, Austria; Chet C. Sherwood, George Washington University, USA Juan Nacher, University of Valencia, Spain; Melissa Holmes, Department of Psychology, University of Toronto, Canada
• Invited talks:	- *Phylogenetic variation of cortical and subcortical "immature" neurons in mammals.* Talk at SINS (Turin, September 2023) in the symposium "Beyond adult neurogenesis: the discovery of natural reservoirs of "immature" neurons for mammalian brain plasticity", chair: Sebastien Couillard-Despres and Luca Bonfanti

• Science communication:	- *L'enigma del neurone giovane.* Conferenza alla Fondazione Faraggiana, Novara, 7 febbraio 2023 (nel ciclo: Il cervello si racconta) - *Plasticità del cervello: del topo, dell'uomo… e degli scienziati*. Darwin Day – Padova 15 febbraio 2023. Conferenza per le scuole secondarie, al mattino (Sede universitaria) e conferenza per pubblico generalista, la sera (Palazzo Moroni) - *Plasticità cerebrale: dal neurone alla vita di tutti i giorni*. Intervento di apertura al convegno ATE (Associazione Traumi Encefalici), Torino 14 ottobre 2023
• Editorial duties:	- Editor in chief *Frontiers in Neurogenesis* - Reviewing editor in *Frontiers in Mammal Science* - Editor of Special Issue in *Int J Mol Sci*: Brain Structural Plasticity: Theme and Variations (with S. Couillard-Despres) 2023
• others	Reports for career advancements of researchers: *Noelia Urban*, IMBA-Austrian Academy of Sciences; *Jon Arellano*, Yale University
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Paolo Marcello Peretto, Full professor (PI)

Supervised PhD students:	Marco Fogli (co-tutor with F. Luzzati)
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	Dustin Penn (Konrad Lorenz Institute of Ethology, Veterinary Medicine University, Vienna); Sylvain Gigan (Laboratoire Kastler-Brossel Sorbonne Université, Paris); Paolo Giacobini (Inserm, UDSL, School of Medicine, Lille, France).
• Invited talks:	
• Science communication:	I Vertebrati: "Un Giorno all'Università" 2022-2023
• Editorial duties:	Associate Editor Frontiers in Neuroscience - Referee for Scientific Journals
• others	SINS 2023- Symposia 9th session, chair "From ontogenesis to physiology and cell replacement: insight into the striatum" 68° Convegno GEI-SIBSC 2023- session 4, chair "neurodevelopment and neurobiology"
Organizational activities and responsibilities at NICO:	Representative of the personnel for safety
Speakers invited:	

Other organizational activities:	
Workshops, Schools or Conferences organized:	Local organizing Committee 20th National Congress of the Italian Society for Neuroscience (SINS)
Technology transfer achievements (patents, etc.):	

Silvia De Marchis, Associate professor, Lead researcher

Supervised PhD students:	Eleonora Dallorto - Ilaria Ghia (co-tutor with S. Bovetti)
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	Michèle Studer, INSERM U636, Nice Sophia Antipolis; Wojciech Krezel INSERM, IGBMC, Strasbourg, France. Christian Schaaf, Institute for Human Genetics, Heidelberg, Germany
• Invited talks:	
• Science communication:	Organization of the 6th Aldo Fasolo Award for communication in neuroscience. Organization of the Workshop Comunicare la scienza: Pillole di storytelling & filmmaking – participation to the roundtable June 8th, 2023, Circolo dei Lettori di Torino.
• Editorial duties:	Reviewing Editor Frontiers in Neurogenesis – Ad hoc Referee for Scientific journals
• others	Member of the evaluation board of the doctoral thesis presented by ELENA MARTÍNEZ RODRÍGUEZ , "Social and sensory deficits in Rett syndrome:neuroanatomical and behavioural analyses in a mouse model deficiente for Mecp2". Valencia, April 25th, 2023
Organizational activities and responsibilities at NICO:	
Speakers invited:	
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:	Organizer and chair of the Symposium "Mechanisms and Therapies of Metabolic And Neurological Disorders" at the 20th SINS Congress, Turin September 16th (label scientifico UIF 2023) Organizer and chair of the Symposium "Mitochondria: Key Players in Neuronal Function and Physiopathology of Neurological Disease" to be held during the FENS in 2024

Technology transfer achievements (patents, etc.):	

Serena Bovetti, Associate professor, Lead researcher

Supervised PhD students:	Ilaria Ghia (co-tutor with Silvia De Marchis)
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	Paolo Giacobini, Lille, France Dustin Penn, Konrad Lorenz Institute, Vienna (Austria) Sylvain Gigan, ENS, Paris (France) Cathie Ventalon, ENS, Paris (France) Bianca Silva, IPMC – CNRS-UCA UMR7275, Sophia-Antipolis, Nice (France)
• Invited talks:	- *Whole-brain representation of imprinted scents* (Società italiana di Neuroetica (SINe), IMT Lucca,11 Maggio 2023) - "*Postnatal paternal exposure shapes neuronal circuits in auditory cortex"* (SINS Conference, Torino, September 15 2023)
• Science communication:	- "*Neurosceinze e musica*" Settimana del cervello, Torino, 14 Marzo 2023 - "*La musica come linguaggio di comunicazione: dall'animale all'uomo*" Alba, 29 Ottobre 2023.
• 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	
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:	-Tommaso Pizzorusso (University of Pisa) -Pascal Hot (Universitè Mont-Blanc Chambery) -Ana Clara Cristovao (University of Beira Interior, Covilhã, Portugal), with Marina Boido and serena Stanga
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Federico Luzzati, Associate professor, Lead researcher

Supervised PhD students:	Marco Fogli (co-supervised with Paolo Peretto)
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	Benedikt Berninger, King's College London, UK; Philip Greulich, University of Southampton, UK Matteo Bergami, University of Cologne, Germany

• Invited talks:	
• Science communication:	Seminario spettacolo “C’era una volta un neurone” in occasione delle manifestazioni “Tram della Scienza” e “Estate a Sud” organizzate dalla associazione Centro Scienza Onlus
• Editorial duties:	
• others	
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Sara Bonzano – PostDoc (Assistant Researcher RTD-A since July 2023)

Supervised PhD students:	Eleonora Dallorto
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	R. Berckervordersandforth and D.C. Lie (FAU - Erlangen, Germany) M. Studer (iBV, UCA, CNRS, INSERM - Nice, France)
• Invited talks:	
• Science communication:	‘First Person’ interviews @ Disease Models & Mechanisms 2023
• Editorial duties:	Referee for: Metabolic Brain Disease Journal of Experimental Neuroscience
• others	
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Stefano Zucca, Marie-Curie fellow

Supervised PhD students:	
Honors, prizes, awards:	Project Support Grant - *British Society for Neuroendocrinology* (5780£)
Outreach activities	
• International collaborations:	Dustin Penn (Konrad Lorenz Institute of Ethology,

	Veterinary Medicine University, Vienna); PSylvain Gigan (Laboratoire Kastler-Brossel Sorbonne Université, Paris); Paolo Giacobini (Inserm, UDSL, School of Medicine, Lille, France).
• Invited talks:	68° Convegno GEI-SIBSC: *"Whole Brain representation of imprinted courtship cues"* SINS 2023: *"Whole-brain representation of imprinted courtship cues"*
• Science communication:	Seminario at CusMiBio, Università degli studi di Milano, Milano, Italy: *"It's Mating Time: i cinque sensi dell'amore"*. Tavola Rotonda Forum Ferdinando Rossi 2023
• Editorial duties:	
• others	
Organizational activities and responsibilities at NICO:	Responsible for the in vivo electrophysiology setup
Speakers invited:	- Vanessa de Luca, Istituto Italiano di Tecnologia, Genova, Italy: *"Gender Dimension in Research"* - Mathew Diamond, SISSA, Trieste, Italy: *"Tactile perception distributed through cortical neuronal populations in rats"* - Valentina Pasquale, Istituto Italiano di Tecnologia, Genova, Italy: *"Neuroscience and Open Science: a winning combination"*
Other organizational activities:	
Workshops, Schools or Conferences organized:	SINS 2023 Symposium Organizer: *"Big Data in Neuroscience: large scale characterization of brain physiology"*
Technology transfer achievements (patents, etc.):	

Alessandra Stella, Postdoc

Supervised PhD students:	
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	Sonja Gruen at Juelich Research Center, Juelich, Germany Bianca Silva at CNRS, Universite' de Nice, France
• Invited talks:	Talk at the Bernstein Conference, in the Workshop "Approaches for analyzing massively parallel neuronal data – Current and future", hosted by the Bernstein Network for Computational Neuroscience, Berlin, Germany. September 2023 - Talk at workshop "Stochastic models of the brain and related

	topics" . Organized by the Department of Mathematics at the University of Torino, Italy. September 2023
• Science communication:	
• Editorial duties:	Reviewer for Frontiers of Computational Neuroscience
• others	-Poster at the Bernstein Conference, hosted by the Bernstein Network for Computational Neuroscience Berlin, Germany. September 2023 -Poster at the SINS Conference Turin, Italy. September 2023
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Ilaria Ghia, PhD Student

Supervised PhD students:	
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	
• Invited talks:	
• Science communication:	Science communication nights "Ricercatori alla Spina" (once a month starting from March 2023). *FameLab Masterclass "Comunicare la ricerca attraverso la parola. Teoria e pratica del talk breve" by L. Alfonsi, FameLab Italia.* 21st April 2023.
• Editorial duties:	
• others	Poster: "*Dissecting the role of the mouse olfactory dopaminergic cells in sexual odor processing*". 20th National Congress of the Italian Society for Neuroscience (SINS). *September 14th-17th, Turin (Italy).* Poster: "*Assessing the role of olfactory dopaminergic cells in processing sexual odors*". FENS Regional Meeting Algarve 2023. *3rd-5th May, Albufeira (Portugal).*
Organizational activities and responsibilities at NICO:	Member of the NICO Green Committee
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Eleonora Dallorto, PhD student

Supervised PhD students:	
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	Michèle Studer (iBV, UCA, CNRS, INSERM - Nice, France)
• Invited talks:	Dallorto E, Bonzano S, Hidisoglu E, Marcantoni A, Sassoè-Pognetto M, Studer M and De Marchis S. 20 h National Congress of the Italian Society of Neuroscience (SINS). 14-17 September, 2023 Turin (Italy). (selected oral presentation by E. Dallorto in the symposium "M*echanisms and therapies of metabolic and neurological disorders" ;Altered morphology of adult-born immature granule neurons and reduced synaptic inhibition in the hippocampal DG of a mouse modeling the neurodevelopmental disorder BBSOAS*;
• Science communication:	Ricercatori alla Spina - Brain Edition (15-17/03/2023); - - Cinemambiente (08/06/2023): presentazione del cortrometraggio realizzato al 6th Swiss Science Film Academy; UNIGHT: la notte della ricerca 30/09/2023; PINT of SCIENCE 2023 (22-23-24 Maggio 2023)
• Editorial duties:	
• others	Poster presentations: Bonzano S. , Dallorto E., , Molineris IvanI., Michelon F., Crisci I., Gambarotta G., Neri F., Oliviero S. , Beckervordersandforth R., Lie Dieter C. , Peretto P., Bovetti S. , Studer M. , De Marchis S. 20 h National Congress of the Italian Society of Neuroscience (SINS). 14-17 September, 2023 Turin (Italy). (poster) "*Nr2f1 shapes mitochondria in the mouse brain unraveling new insights into the neurodevelopmental disorder BBSOAS*". Dallorto E, Bonzano S, Hidisoglu E, Molineris I, Gambarotta G, Marcantoni A, Studer M, and De Marchis S. JEDNs 2023, Journées de l'Ecole Doctorale de Nice - 15-17 May, 2023 Nice (France). (poster) ;*Nr2f1 haploinsufficiency leads to reduced inhibition of hippocampal DG neurons and mitochondrial dysfunction in the adult mouse brain*.
Organizational activities and responsibilities at NICO:	
Speakers invited:	
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:	
Technology transfer achievements (patents, etc.):	

Marco Fogli, PhD student

Supervised PhD students:	
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	Philip Greulich (University of Southampton)
• Invited talks:	
• Science communication:	"Just Neuroblast - Disponibili nuovi neuroni on demand". Ricercatori alla spina – Brain edition. Offtopic, Turin, 15 marzo 2023.
• Editorial duties:	na
• others	Oral presentation: "*Widespread and continuous astrocytes activation support long-term neurogenesis in the lesioned striatum*". 20th National Congress of the Italian Society for Neuroscience (SINS) - Symposium 5_1: Beyond adult neurogenesis: reservoirs of "immature" neurons for brain plasticity. Turin (Italy), 14-17 September 2023. Poster presentation and participation: - M. Fogli, G. Nato, P. Greulich, J. Pinto, P. Peretto, A. Buffo and F. Luzzati. Widespread and continuous astrocytes activation support long-term neurogenesis in the lesioned striatum. "16th European Meeting on Glial Cells in Health and Disease". Berlin (Germany), 8-11 July 2023. - S. De Vreese, F. Luzzati, M. Fogli, C. Corona, C. Palmitessa, M. André, S. Mazzariol. Investigating sensitivity: a three-dimensional reconstruction of striped dolphin's external ear canal neural network. "Oceanois 2023". Barcelona (Spain), 22-26 May 2023. Membership of Scientific Societies: Italian Society of Neuroscience (SINS)
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

Marco Ghibaudi, Postdoc

Supervised PhD students:	na
Honors, prizes, awards:	"Mariella Graffi" Award 2023 (Accademia dei Lincei) for best master thesis in Comparative Anatomy

Outreach activities na	
• International collaborations:	Chet Sherwood (George Washington University, Washington DC, USA); Bruno Benedetti, Sebastien Couillard-Despres (Institute of Experimental Neuroregeneration, Paracelsus Medical University, Salzburg, Austria)
• Invited talks:	na
• Science communication:	na
• Editorial duties:	na
• others	Poster: Pattaro A., Ghibaudi M., Bonfanti L. *Distribution and density of DCX-positve "immature" neurons in Beagle dogs.* 20th National Congress of the Italian Society of Neuroscience (SINS). 14-17 September, 2023 Turin (Italy). Pattaro A., Ghibaudi M., Corrente C., Bonfanti L. *Immature neuron distribution and density in dogs.* GISN 2023. 24-25 November, 2023 Verona (Italy)
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

Alessia Pattaro, PhD student

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	Chet C. Sherwood, George Washington University, USA
• Invited talks:	Pattaro A., Ghibaudi M., Corrente C., Bonfanti L. *"Immature neuron distribution and density in dogs".* XXXIII National Congress of the Italian Group for the Study of Neuromorphology (GISN). 24-25 November 2023, Verona (Italy).
• Science communication:	na
• Editorial duties:	na
• others	Poster presentation: Pattaro A., Ghibaudi M., Bonfanti L. *"Distribution and density of DCX-positive "immature" neurons in Beagle dogs*". 20th National Congress of the Italian Society for Neuroscience (SINS). *14-17 September 2023, Turin (Italy).*

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

4. Research activity in 2023

a. Summary (500 characters)

Different aspects of postnatal/adult brain plasticity in health and pathology 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, and their *trade-off* from small-brained to large-brained species
iii) the effect of Nr2F1 haploinsufficiency on the adult hippocampus
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, both in neurogenic niches and in the parenchyma, but also 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.
2) How different types of plasticity (AN versus "immature" neurons) are phylogenetically distributed among mammals? How the immature neurons behave at different ages? 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., neurogenesis without division).
3) How widespread is the latent neurogenic potential of the parenchyma, what is the fate of adult neural stem cells (NSCs) and how is regulated the identity and function of adult born neurons in physiological or pathological conditions?
The rationale of the research carried out in 2023 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 (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. Our previous research has shown that the transcriptional regulator Nr2f1 is highly expressed in the adult mouse hippocampal dentate gyrus (DG), where it plays a crucial role in directing progenitors towards a neuronal fate. 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.

3.2 We demonstrated that in response to injury, some astrocytes in the striatal parenchyma reawaken a latent neurogenic potential. However, the prevalence, spatial distribution and dynamics of these ectopic NSCs were not resolved. Consequently, the mainstream view in the field still adheres to the idea that NSCs represent rare cells confined to the canonical neurogenic niches. To probe the neurogenic potential of the parenchyma in the last years we the analysis of the spatio-temporal dynamics of striatal astrocytes neurogenic activation after excitotoxic lesions. In parallel, we have also completed the analysis of the fate and integration capacity of the newborn neurons generated after lesion.

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 role of the transcriptional regulator Nr2f1 on AN in the DG

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. Using a combination of iDISCO tissue clearing and light-sheet fluorescence microscopy, we performed whole brain imaging of cFos-stained neurons in female mice acutely exposed to odors from familiar and unfamiliar males. We then evaluated the number of cFos-positive cells and their fluorescence intensity in all brain areas using the ClearMap software. We interpreted the number of activated cells as a proxy for the degree of activation of a given area during odor presentation. We identified functional subnetworks by constructing undirected graphs of brain areas that were differentially activated across experimental groups. For generalization and ecological validation of our results on laboratory female mice, we applied this analytical pipeline to the study of wild mice populations. Our results on both laboratory and wild animals, suggest that amygdalar and hypothalamic areas

may be crucial in the neural circuits involved in sexual imprinting in the mouse brain. We are now applying in vivo calcium imaging using Miniscope technology directly on identified areas.
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. We studied the amount of cortical immature neurons in the murine piriform cortex at different ages, showing a remarkable reduction from young to old stages, while such a decrease was far less evident in gyrencephalic species. Nevertheless, even in old rodents, the small population of immature neurons do awake and fully mature as new functional neurons. These findings suggest an evolutionary developmental mechanism for plasticity in large brains, granting a reservoir of young cells for the cerebral cortex.
iii) Nr2f1 function in neurons. Mitochondrial dysfunction is implicated in neurodevelopmental disorders; whether and how Nr2f1 can regulate mitochondria function in neural cells is still completely unknown. we identified, by genome-wide and in silico analyses, a set of nuclear-encoded mitochondrial genes as potential genomic targets under direct NR2F1 transcriptional control in neurons. We demonstrated that conditional NR2F1 loss of function within the adult mouse hippocampal neurogenic niche results in a reduced mitochondrial mass associated with mitochondrial fragmentation in newborn neurons. Importantly, we also found dysregulation of several nuclear-encoded mitochondrial genes and downregulation of key mitochondrial proteins in the brain of Nr2f1-heterozygous mice, a validated BBSOAS model. Our data point to an active role for NR2F1 in the mitochondrial gene expression regulatory network in neurons and support the involvement of mitochondrial dysfunction in BBSOAS pathogenesis.
iv) Lesion induced striatal neurogenesis. Our results indicate that the 1) The striatal astrocytes include a widespread population of dormant NSC; 2) in response to injury single astrocytes stochastically enter an activated state generating clones of transit amplifying progenitors that generate neuroblasts; 3) activation events occur at a constant rate but in random locations, preferentially far from previous events; 4) in contrast to canonical niches the great majority of parenchymal astrocytes come back to quiescence after activation and are not depleted. We demonstrate a widespread neurogenic potential of striatal astrocytes, and a global permissiveness of striatal parenchyma. 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*: Our interdisciplinary approach, combining tissue clearing, advanced imaging techniques, and network analysis allowed to identify the brain circuits recruited by exposure to male olfactory cues in laboratory and wild mice populations.

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 function in neurons.* Our data point to mitochondrial dysfunction in neural tissue as a potential key pathological mechanism in BBSOAS, suggesting that the current estimation of mitochondrial involvement in BBSOAS patients might be underestimated.

iv) *Lesion induced striatal neurogenesis*: We demonstrated an unprecedented level of neurogenic potential within the mature brain parenchyma, comparable to that found in canonical niches. Furthermore, we have shown that lesion-induced striatal neurogenesis generates a specific transient cell type that integrate in pre-existing circuits and may play a role in compensatory plasticity after lesion.

f. Publications

1. Ghibaudi M, Amenta A, Agosti M, Riva M, Graïc J-M, Bifari F, Bonfanti L (2023) Consistency and variation in doublecortin and Ki67 antigen detection in the brain tissue of different mammals, including humans. International Journal of Molecular Sciences 24, 2514.
Research article – Q1

2. Ghibaudi M, Marchetti N, Vergnano E, La Rosa C, Benedetti B, Couillard-Despres S, Farioli-Vecchioli S, Bonfanti L (2023) Age-related changes in layer II immature neurons of the murine piriform cortex. Frontiers in Cellular Neuroscience 17:1205173.
Research article – Q1

3. Benedetti B, Reisinger M, Hochwartner M, Gabriele G, Jakubecova D, Benedetti A, Bonfanti L, Couillard-Despres S (2023) The awakening of dormant neuronal precursors in the adult and aged brain. Aging Cell 30:e13974.
Research article – Q1

4. Bonfanti L, La Rosa C, Ghibaudi M, Sherwood CC (2023) Adult neurogenesis and "immature" neurons in mammals: An evolutionary trade-off in plasticity? Brain Structure & Function (ePub, October 13, 2023)
Review – Q1

5. Bonfanti L, Couillard-Després S (2023) Neuron and Brain Maturation 2.0. International Journal of Molecular Sciences 2*4*(23), 17113
Editorial – Q1

6. Bonzano S, Dallorto E, Molineris I, Michelon F, Crisci I, Gambarotta G, Neri F, Oliviero S, Beckervordersandforth R, Lie DC, Peretto P, Bovetti S, Studer M, De Marchis S. NR2F1 shapes mitochondria in the mouse brain, providing new insights into Bosch-Boonstra-Schaaf optic atrophy syndrome. Disease Model and Mechanisms 16(6):dmm049854.
Research article – Q1

7. Scandiffio R, Bonzano S, Cottone E, Shrestha S, Bossi S, De Marchis S, Maffei ME, Bovolin P (2023) Beta-Caryophyllene modifies intracellular lipid composition in a cell model of hepatic steatosis by acting through CB2 and PPAR receptors. International Journal of Molecular Sciences 23;24(7):6060.
Research article – Q1

8. Zucca S, Puche AC, Bovetti S (2023) The neural circuitry of mating behaviors. Frontiers in Neural Circuits. 16:1102051.
Editorial – Q2

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: a new molecular regulator of mitochondrial function in adult born neurons, the activation of parenchymal astroglia NSC potential after injury, the comparative approach to phylogenetic variation of non-newly generated "immature" neuronal populations in mammals and 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 and MSCA 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 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 dormant neurogenic potential of the parenchyma) 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 and MSCA research projects). 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 progenitor populations, 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.* By using an interdisciplinary approach that combines tissue clearing, advanced imaging techniques, and network analysis we identified the brain circuits recruited by exposure to male olfactory cues in laboratory and wild mice populations (this latter part has been performed in collaboration with Prof. Dustin Penn, University of Vienna).We are now applying the Miniscope technology to target the regions previously identified, and image in vivo the 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 high synchrony in the activation of the QA induced neurogenic response between animals will be exploited to analyze at the cellular molecular level the changes in the environment and astrocytes population associated with neurogenic activation. To this aim we will use both bulk, single cell RNAseq and spatial transcriptomics to identify specific genes and cell populations associated with the activation of the neurogenic potential. Particular emphasis will be devoted to identifying pathways associated with the acquisition of primed or competent states, as observed in other stem cell systems. Selected markers of specific cell states will be further analyzed in the tissue through multiplexing immunohistochemistry and 3D reconstructions in combinations with exogenous birth dating markers such as BrdU, EdU or Ki67-CreERT2 lineage tracing. In parallel, we will perform similar analyses, in collaboration with Annalisa Buffo at NICO, in mice in which we will abrogate the expression of the transcription factor SOX2. Preliminary results indicate that mosaic deletion of this TF in astrocytes can abrogate the initiation of the neurogenic response through both cell autonomous and non-cell autonomous factors. This screening will allow us to identify factors involved in the activation of the parenchymal latent neurogenic potential. 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 (neurogenesis without division), and including progenitor specification, hormone-linked behavior, lesion-induced repair, and the evolutionary trade-off in plasticity. 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 canonical neurogenic sites and parenchyma 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): please fill-out this section only in the case of innovative technologies

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 at different postnatal ages 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, we aim to develop a high-throughput imaging technology based on multimodal optical fibers integrated in a wire-free head-mounted device. We already tested this approach in vivo, and, with further implementation, we aim in recording network activity from multiple brain regions with single-unit resolution, low invasiveness, and in freely moving animals.
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 2023

Laboratory name: Physiopathology of neural stem cells

1. LABORATORY DESCRIPTION – PERSONNEL:

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 for research on oligodendroglial physiopathology.

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

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

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

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

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

Giacomo Turrini, PhD candidate, MSc in Biotechnology for Neuroscience, 23/06/1998, +393661885920, giacomo.turrini@unito.it. Investigation of cerebellar astrocyte heterogeneity through *in silico* and imaging techniques.

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

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

Maryam Khastkhodaei Ardakani, PhD candidate, MSc in Anatomical Sciences, 30-06-1991, +390116706632, maryam.khastkhodaeiardakani@unito.it. Pharmacological and cell transplantation approaches to promote recovery in microcephaly 17 (MCPH17) models.

Anna Incerti Tinterri, Research Fellow, MSc in Medical Biotechnology, 16-05-1998, +393404081253, anna.incertitinterri@unito.it. Targeting oligodendroglial cell dysfunction to treat

cognitive defects and epilepsy in autosomal recessive primary microcephaly-17 (MCPH17) models.

Niccoló Di Cintio, Research Fellow, MSc in Neurobiology, 16-11-1992, +393455998634, niccolo.dicintio@unito.it. Behavioral characterization of conditional Citron kinase mutants.

2. CURRENT GRANTS

Starting-end date	Project Title and ID	Beneficiary	Funding Program/Agency	Role of the unit	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
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 and Valentina Cerrato	Banca d'Italia	Coordinator (PI)	25,000 €	FCO
2023-2024	In vitro and in vivo molecular mapping of cell therapy for	Annalisa Buffo	PRIN2022, MUR	PI of research unit	98.405 €	UNITO

	Huntington Disease at single-cell resolution					
2023-2025	Targeting oligodendrocyte dysfunctions to treat cognitive defects and epilepsy in primary autosomal recessive microcephaly-17 (MCPH17) models (ID: GMR22T1066)	Enrica Boda	Telethon Foundation Multiround 21-24 – Round 1 2022 Track Basic	PI	129,500 €	Telethon
2023-2025	Role of interleukin 6 in the pathogenesis of Rett syndrome: focus on astrocyte-neuron crosstalk and its therapeutic implication (ID: P20225Z3J5)	Enrica Boda	PRIN2022-PNRR, MUR	PI of the research unit	100,000 EUR	UNITO
2023-2025	Targeting glial cell dysfunctions to treat cognitive defects and epilepsy in primary	Enrica Boda	PRIN2022, MUR	PI	90,000	UNITO

	autosomal recessive microcephaly-17 (MCPH17) models (ID: 20224YJBBP)					

3. SCIENTIFIC ACTIVITIES IN 2023

Annalisa Buffo, PI

Supervised PhD students:	Maryam Khastkhodaei (co-supervised with E. Boda) Marta Ribodino, Giacomo Turrini (co-supervised with Valentina Cerrato)
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:	*Glia precursor cells heterogeneity and risk for disease*, invited Lecture for the Neuroscience Day, University of Lund, May 4, 2023; *Reconstruction of striatal circuits and promotion of functional recovery in Huntington's Disease: advancement in cell-based approaches*, invited speaker in Symposium; Meeting of the Italian Society for Neuroscience SINS, Turin, September 5, 2023; *Cell based approaches to reconstruct striatal circuits and promote functional recovery in a rat model of Huntington's Disease: challenges and advances;* invited speaker, 33rd Annual Meeting of the Network for European CNS Transplantation and Restoration, Naples, October 25, 2023.
● Science communication:	UNISTEM Day Torino 2023 – organizer - https://www.nico.ottolenghi.unito.it/Scuole/UniStem-Day-Giornata-Staminali/UniStem-Day-2023-Tecnologia-che-ebbrezza-e-la-scienza-bellezza BRAIN AWARENESS WEEK (La Settimana del Cervello) – organizer - https://www.nico.ottolenghi.unito.it/Neuroscienze-per-voi/Settimana-del-Cervello/Dal-14-al-17-marzo-torna-la-Settimana-del-Cervello
● Editorial duties:	Member of the Glia Editorial Board Ad hoc reviewer for the following journals in 2023:

	Glia, Molecular Therapy, Nature Neuroscience, Stem Cell Research and Therapy, Cells, Stem Cells
• others	Evaluation panel member for the French High Council for Evaluation of Research and Higher Education (Hcéres) of the ICM - Institut du cerveau et de la moelle épinière, Paris, (2023). Evaluating member for tenure-eligible lecturers in Physiology, University of Barcelona, ES (2023). Evaluating member for non-tenure track researcher, University of Milan. Evaluating member for the Promotion of Associate Professors to Full Professors, Friedrich-Alexander-Universität Erlangen-Nürnberg, DE. Grant reviewer for the following funding agencies in 2023: Swiss National Science Foundation; Intermediate Project Grants - 2023, RoseTree Trust; Fédération pour la Recherche sur le Cerveau; Brain Research UK; Spanish Ministry of research and innovation. Membership in Scientific Societies in 2023: Federation of the European Neuroscience Societies (FENS) Italian Society for Neuroscience (SINS) International Society for Stem cell Research (ISSCR) Society For Research On The Cerebellum And Ataxias (SRCA) Attended meetings: XVI European Meeting on Glial Cells in Health and Disease, Berlin, July 8-11, 2023 SINS, NECTAR 2023 Conference, 23-25 October 2023, Naples, IT Annual meeting of the NSC-Reconstruct Consortium, April 2023, Bellagio, Italy. Invited for interview, ERC Synergy Grants 2023
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:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	Workshop on: Transplant connectomics: Strategies to improve the structural and functional integration of replacement cells, NSC-Reconstruct Consortium, January 25-26th, 2023.
Technology transfer achievements (patents, etc.):	

Enrica Boda, Lead Responsible of Research on oligodendroglia and myelination in vivo

Supervised PhD students:	Maryam Khastkhodaei (co-supervised with A Buffo); Martino Bonato
Honors, prizes, awards:	-
Outreach activities	
● International collaborations:	-
● Invited talks:	XX Meeting of the Italian Society of Neuroscience (SINS), Turin, 16 Sept 2023. *Molecular and functional heterogeneity in dorsal and ventral oligodendrocyte progenitor cells of the mouse forebrain in response to DNA damage.* XVI European Meeting on Glial Cells in Health and Disease, Berlin (Germany), 11 July 2023. *Molecular and functional heterogeneity in dorsal and ventral oligodendrocyte progenitor cells of the mouse forebrain in response to DNA damage.* FENS Regional Meeting, Algarve (Portugal), 3-5 May 2023. *Molecular and functional heterogeneity in dorsal and ventral oligodendrocyte progenitor cells of the mouse forebrain in response to DNA damage.*
● Science communication:	Pint of Science - Beautiful Mind, May 24th 2023. Keep s-myelin'! Plasticità della mielina nell'adulto. Serata di divulgazione scientifica al Birrificio Torino. Plasticità della mielina nel cervello adulto: substrato dell'apprendimento e della memoria? Articolo divulgativo sul sito istituzionale del Neuroscience Institute Cavalieri Ottolenghi, pubblicato in occasione del World Brain Day The Green Brain: un "Caffe Scientifico" per comprendere l'impatto di alimenti e inquinanti ambientali sulla salute del nostro cervello (Evento satellite della Notte Europea dei Ricercatori UNight 2023). Sept 30th 2023. Casa del Quartiere, San Salvario, Torino Penne Amiche della Scienza (corrispondenza ed incontri con la 2A della scuola media di Cerea, VR): https://sites.google.com/view/penne-amiche-della-scienza Reviewer for the Giovedì Scienza Award
● Editorial duties:	Ad-hoc Reviewer (approx. 12 review/year) for: Nat Commun, Glia, Progress in Neurobiology, Advanced Science, Front Neurosci, Sci Rep, Mech Ageing Dev, Mol Neurobiol, Eur J Neurosci, SpringerNature NeuroMethods book series, etc.
● others	Grant Reviewer (2023) for: Italian Foundation Multiple Sclerosis (FISM) PhD Thesis reviewer:

	Dr. Ana Cristina Ojalvo Sanz - Progeny and Cell Potential of NG2-progenitors. Programa de Doctorado en Neurociencia, Universidad Autonoma de Madrid, Spain Membership in Scientific Societies: Federation of the European Neuroscience Societies (FENS) European Society of Neurochemistry (ESN) Italian Society of Neuroscience (SINS) Italian Society of NeuroImmunology (AINI) BraYn (Brainstorming Research Assembly for Young Neuroscientists) Association Founder of the *Italian Glia Network* (IGN, with V. Cerrato – Unito; C. Falcone – SISSA, Trieste; G. Losi – UniFe; L. Civiero – UniPd; N. Iraci – UniCt; F. Petrelli - UniLausanne): https://italianglianetwork.wixsite.com/italian-glia-network/ Member of the "Conseil de Perfectionnement" of the Master Program in Neuroscience, Université de Paris, France (https://master-neuroscience-paris.fr/) Attended meetings: EMBL in Italy, Turin, May 18th-19th FENS Regional Meeting, Algarve (Portugal), 3-5 May 2023 XVI European Meeting on Glial Cells in Health and Disease, Berlin, July 8-11, 2023 XX Meeting of the Italian Society of Neuroscience (SINS), Turin, 16 Sept 2023 NECTAR 2023 Conference, 23-25 October 2023, Naples, IT
Organizational activities and responsibilities at NICO:	Member of the "Public Engagement Committee" and "Green Policies Committee" of the NICO Responsible for the Histology Lab; Organizer of the annual Plogging walk in the frame of M'Illumino di Meno Day
Speakers invited:	Wenhui Huang (Universität des Saarlandes Homburg, Germany) Marco Cambiaghi (University of Verona, Italy)
Other organizational activities:	-
Workshops, Schools or Conferences organized:	Symposium: *"Cell intrinsic and extrinsic regulation of oligodendrocyte biology and re-/myelination"*, XX Meeting of the Italian Society of Neuroscience (SINS), Turin, 14-17 Sept 2023. Symposium: *"Oligodendrocyte progenitor cell fates and interactions with neurons in the adult and developing brain"*, XVI European Meeting on Glial Cells in Health and Disease, Berlin, 8-11 July 2023. Member of the Scientific Committee of BraYn (*Brainstorming Research Assembly for Young Neuroscientists)* (https://www.braynconference.com/). Chair of the *Neurophysiology and Neural Plasticity* session (with Rosa Paolicelli, Univ. Lausanne & Giovanna Calabrese, UniMe)

Technology transfer achievements (patents, etc.):	na

Gabriela B. Gomez Gonzalez, PostDoctoral Fellow

Supervised PhD students:	na
Honors, prizes, awards:	
Outreach activities	
● International collaborations:	NSC-Reconstruct network (E.Cattaneo, University of Milano), Malin Parmar (University of Lund). Ataulfo Martinez (Universidad Nacional Autonoma de Mexico)
● Invited talks:	Talk (selected from poster) at SINS 2023, To, It. "Striatal calcium signals from WGE allografts in a rat model of Huntington's Disease". Gómez-González GB, Parolisi R, Ribodino M, Aghaei K, Zucca S, de'Sperati C, Buffo A.
● Science communication:	
● Editorial duties:	
● others	Mentoring: Biological Science Bachelor degree; Student mentoring. Alice Mollo (Jan-Dec 2023) (Co-tutoring with Enrica Boda). Membership in Scientific Societies in 2023: Italian Society of Neuroscience (SINS) Attended Congress: -33rd Annual meeting of the Network for European CNS Transplantation and Restoration (NECTAR). Naples, Italy. Oct 23-25th. *Poster presentation* -SINS Annual Meeting. Turin, Italy.Sept 14th-17th. *Talk (selected from poster).* -NSC-Rec Annual Meeting. Bellagio, Italy. April 11-13th. *Poster presentation*
Organizational activities and responsibilities at NICO:	Part of the NICO Green-Committe.
Speakers invited:	
Other organizational activities:	Internal Buffo Lab-meetings (organization- moderation)
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Giulia Nato, Post-doctoral Fellow

Supervised PhD students:	na
Honors, prizes, awards:	Best Poster Award National Congress of the Italian Society for Neuroscience SINS 2023
Outreach activities	

• International collaborations:	Benedikt Berninger (King's Colledge; London), Magdalena Goetz (University of Munich)
• Invited talks:	-
• Science communication:	C'era una volta un neurone…Storie di un groviglio chiamato cervello. In collaborazione con Associazione Centroscienza onlus. 14/06/2023
• Editorial duties:	-
• others	Membership in Scientific Societies in 2023: Italian Society of Neuroscience (SINS) Attended meetings: -SINS, September 14-17, 2023- *poster presentation* -XVI European Meeting on Glial Cells in Health and Disease, Berlin, July 8th-11th, 2023 - *Poster presentation*
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Martina Lorenzati, Post-doctoral Fellow

Supervised PhD students:	na
Honors, prizes, awards:	-
Outreach activities	
• International collaborations:	Fernando De Castro (Cajal Institute, Madrid, Spain) Sabah Mozafari (CNRS, University of Paris, France)
• Invited talks:	*Modeling ADLD pathology with human iPS-derived glial cells: altered phenotypes and rescue strategies* - September 16th, XX National Congress of the Italian Society for Neuroscience (SINS), September 14th-17th 2023, Turin, Italy
• Science communication:	*Ricercatori alla Spina - Brain Edition*, March 17th and May 18th 2023 *Pint of Science - Beautiful Mind*, May 22nd-24th 2023
• Editorial duties:	-
• others	Membership in Scientific Societies in 2023: Italian Society of Neuroscience (SINS) Federation of European Societies (FENS) Attended meetings: EMBL in Italy, Turin, May 18th-19th - *Poster presentation* XVI European Meeting on Glial Cells in Health and Disease, Berlin, July 8th-11th, 2023 - *Poster presentation* SINS, September 14th-17th, 2023 - *Oral presentation*
Organizational activities and responsibilities at NICO:	Co-Responsible of the Dissection Room

Speakers invited:	-
Other organizational activities:	Co-organizer of *Pint of Science - Beautiful Mind*, May 22nd-24th 2023
Workshops, Schools or Conferences organized:	Proponent and chairperson of the Symposium "*Stem cell meet glial cells: human iPS-derived glia to study myelin diseases*" - XX National Congress of the Italian Society for Neuroscience (SINS), September 16th 2023, Turin, Italy
Technology transfer achievements (patents, etc.):	

Roberta Parolisi, Post-doctoral Fellow

Supervised PhD students:	na
Honors, prizes, awards:	-
Outreach activities	
● International collaborations:	NSC-Reconstruct network (E Cattaneo, University of Milano; A Bosio, Miltenyi Biotec)
● Invited talks:	
● Science communication:	
● Editorial duties:	Ad hoc reviewer for Neurochemical Research, Journal of the Neurological Sciences and Frontiers Aging Neuroscience.
● others	Membership in Scientific Societies in 2023: Italian Society of Neuroscience (SINS) Attended meetings: Annual meeting of the NSC-Reconstruct Consortium, April 2023, Bellagio, Italy - *poster presentation* SINS, September 14-17, 2023- *poster presentation*
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:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Valentina Cerrato, Post-doctoral Fellow

Supervised PhD students:	Giacomo Turrini (co-supervised with Annalisa Buffo)
Honors, prizes, awards:	Early Career Scientist Grant to attend the XVI European Meeting on Glial Cells in Health and Disease, Berlin, 2023, awarder by Hello Bio Ltd
Outreach activities	
● International collaborations:	Prof. Ludovic Telley (University of Lausanne, Switzerland); Prof. Magdalena Götz (University of Munich, Germany)
● Invited talks:	*The multiple levels of cerebellar astrocytes heterogeneity: from developmental trajectories, to spatial patterning and functional*

	interactions with specific neuronal circuitries, 19 Jan 2023, "Astrocyte cafè" (online webinar series) *Unveiling the diversity of cerebellar astrocytes: insights into their molecular identities, development and functions*, 16 Sept 2023, XX National Congress of the Italian Society for Neuroscience, Turin, September 14-17, 2023. *Unveiling the diversity of cerebellar astrocytes: insights into their molecular identities, development, and functions in relation to cerebellar neuronal circuitries*, SIF Mini Symposium on Microglia and Astrocytes crosstalk with neurons in physiological and pathological conditions (online), November 3, 2023.
• Science communication:	Ricercatori alla Spina - Brain Edition, March 17th 2023 and May 18th 2023 *Pint of Science - Beautiful Mind*, May 22nd-24th 2023
• Editorial duties:	Review editor for Frontiers in Neuroscience (section Neurogenesis) Ad hoc reviewer for the following journals in 2023: Frontiers in Cellular Neuroscience (x2), Glia, Nature Neurosci (co-reviewer with Prof. Luca Bonfanti).
• others	Co-Founder of the Italian Glia Network (IGN) Membership in Scientific Societies in 2023: Federation of the European Neuroscience Societies (FENS) Italian Society of Neuroscience (SINS) International Society for Neurochemistry (ISN) ALBA Network Attended meetings: XVI European Meeting on Glial Cells in Health and Disease, Berlin, July 8-11, 2023 73rd SIF National Congress, September 6-8, 2023 SINS, September 14-17, 2023
Organizational activities and responsibilities at NICO:	- Responsible of the ZEISS Axio Scan.Z1 use at NICO - Responsible of the Neurolucida system II
Speakers invited:	na
Other organizational activities:	Co-organizer of *Pint of Science - Beautiful Mind*, May 22nd-24th 2023
Workshops, Schools or Conferences organized:	Chairperson and proponent of the symposium: "From development to disease – how astrocyte heterogeneity sculpts the brain" in the frame of the XX National Congress of the Italian Society for Neuroscience (SINS), 16 September 2023, Turin, Italy
Technology transfer achievements (patents, etc.):	

Anna Incerti Tinterri, Research Fellow

Supervised PhD students:	na
Honors, prizes, awards:	
Outreach activities	
● International collaborations:	
● Invited talks:	
● Science communication:	
● Editorial duties:	
● others	Courses: - "BIOLOGIA E GESTIONE DEGLI ANIMALI DA LABORATORIO, MODULI 3.1, 4, 5, 6.1, 7. DM 5 AGOSTO 2021 RODITORI E LAGOMORFI "released by Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (Izsler). - "OPBA: FORMAZIONE PER I COMPITI, MODULI 25, 50, 51 " released by Izsler. - “ZEBRAFISH COME ORGANISMO MODELLO: APPROCCI SPERIMENTALI IN VITRO E IN VIVO NELLA RICERCA SCIENTIFICA ” released by Izsler. - "ETICA E CONCEZIONE DEI PROGETTI, MODULI 9, 10, 11, DM 5 AGOSTO 2021" released by Izsler. - “LEGISLAZIONE NAZIONALE ED ETICA LIVELLO 1, MODULI 1 E 2, DM 5 AGOSTO 2021” released by Izsler. - How to Make & Present a Poster for Neuroscience 2023, Sep 18, 2023, released by Society for neuroscience. Meetings and conferences: - BraYn 2023-sixth brainstorming research assembly for young neuroscientists, September 27th-29th 2023, Naples, Italy. (Poster presentation); - ICOCIMS: International student Congress of Clinical Innovation and Medical Sciences, 9-11 Nov 2023.
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Giacomo Turrini, PhD Student

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

• International collaborations:	Prof. Ludovic Telley (University of Lausanne, Switzerland)
• Invited talks:	
• Science communication:	
• Editorial duties:	
• others	Meetings and conferences: - DNF Symposium 2023, 15 September 2023, Lausanne, CH - Webinars and workshops: - NICO NeuroWebinar - on fridays, at 2.00 p.m.- https://www.nico.ottolenghi.unito.it/Agenda/NICO-NeuroWebinar; - Public engagement and outreach - Toolbox Course, *17h-18th November 2023;*
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Marta Ribodino, PhD student

Supervised PhD students:	na
Honors, prizes, awards:	Best poster presentation award at the NECTAR 2023 Conference, 23-25 October 2023, Naples, IT
Outreach activities	
• International collaborations:	Prof. Malin Parmar and prof. Tomas Bjorklund (Lund University, Sweden); Prof. Elena Cattaneo (University of Milano)
• Invited talks:	
• Science communication:	Ricercatori alla Spina - Brain Edition, March 17th 2023
• Editorial duties:	
• others	Membership in Scientific Societies in 2023: Italian Society of Neuroscience (SINS) ALBA Network Attended meetings: NECTAR 2023 Conference, 23-25 October 2023, Naples, IT - *poster presentation* SINS, September 14-17, 2023 - *poster presentation* Annual meeting of the NSC-Reconstruct Consortium, April 2023, Bellagio, Italy - online. Neuroscience Day 2023. May 4th, Lund (Sweden) Segerfalk Symposium 2023. May 1st, Lund (Sweden) 2-month Erasmus Traineeship period in prof. Malin Parmar and prof. Tomas Bjorklund's labs

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:	
● Science communication:	Ricercatori alla spina - Brain edition, March 15th and May 18th 2023; The Green Brain (U*Night - La Notte Europea dei Ricercatori e delle Ricercatrici 2023), September 30th 2023.
● Editorial duties:	
● others	Course: - Open Science A to Z, April 17-18 2023 Webinars and Workshops: - “The lung microbiome regulates brain autoimmunity”, ESNI Journal Club with Authors, January 31st 2023 - "Modern information technologies for evaluation of health risk in extreme environments", PhD seminar, April 28th 2023 - “non coding RNA in axon guidance, PhD seminar, June 6th 2023 - “Mechano-biology of tumor microenvironment”, PhD Seminar, June 7th 2023 - "Transposable Elements - Jumping Genes", PhD seminar, July 3rd 2023 - "Advances in AI-based approaches to cancer imaging and molecular profiling", PhD workshop, July 4th 2023 - "Modern information technologies for evaluation of health risk in extreme environments", PhD seminar, July 14th 2023 - “How Does Myelin Contribute to Brain Plasticity?”, Society for Neuroscience webinar, December 7th 2023 Attended Meetings: - EMBL in Italy 2023, May 18-19 2023 - “Onconephrology: the new challenge for nephrologists and oncologists”, May 25-26 2023 - 16th European Meeting on Glial Cells in Health and Disease- July 8th-11th 2023, Berlin, Germany. (Poster presentation)

	- SINS PhD meeting 2023- Italian Society for Neuroscience - September 14th, 2023, Turin, Italy. - 20th National Congress of the Italian Society for Neuroscience (SINS)- September 14th-17th, 2023, Turin, Italy. (Poster presentation)
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Maryam Khastkhodaei Ardakani, PhD student

Supervised PhD students:	Not applicable
Honors, prizes, awards:	
Outreach activities	
● International collaborations:	
● Invited talks:	
● Science communication:	
● Editorial duties:	
● others	Course - The Introductory Course on Glial Cell Biology 2023- H.Kettenmann, A.Faissner, L.Dimou, C.Klämbt, C.Jakob, H.Neumann, V.Siffrin, S.Wolf, K.Biber, B.Berninger - held by Glia 2023 conference, Berlin, Germany. July 7th 2023 (10 a.m. to 5 p.m.) Seminars, webinars and workshops: - Choroid plexus: a new entry point to study the brain development- Annarita Patrizi- PhD's Seminar Cycle. May 30th 2023, Institute of Anatomy, Turin. - European microglia webinar series- Monthly webinars on microglial research- https://www.microglia.info/ - Astrocyte Café - Series of monthly meetings about astrocyte research - NICO NeuroWebinar - periodically at 2.00 p.m.- https://www.nico.ottolenghi.unito.it/Agenda/NICO-NeuroWebinar Meetings and conferences: - Fens Regional Meeting- May 3rd-5th 2023, Albufeira, Portugal. (Poster presentation) - The Cambridge Centre for Myelin Repair (CCMR)'s 2nd Annual Symposium- May 25th-26th 2023. (Online event) - 16th European Meeting on Glial Cells in Health and Disease- July 8th-11th 2023, Berlin, Germany. (Poster presentation)

	- SINS PhD meeting 2023- Italian Society for Neuroscience - September 14th, 2023, Turin, Italy. (Poster presentation) - 20th National Congress of the Italian Society for Neuroscience (SINS)- September 14th-17th, 2023, Turin, Italy. (Poster presentation) - BraYn 2023-sixth brainstorming research assembly for young neuroscientists, September 27th-29th 2023, Naples, Italy. (Poster presentation) others: - Discussant/Peer advisor of PhD student Dr. Eleonora Dallorto- Data report of PhD Students (37th cycle)- 15th February 2023, Turin, Italy. Public Engagement-related activity: - Online training for presenting PhD research work in 3 minutes in an easy-to-understand way to a non-specialist audience in ENGLISH- Held by UNITA, April-June 2023. (7 hours) - Awarded the 3rd place in "My three-minute PhD thesis" local competition- June 28th 2023, Turin, Italy. Membership in Scientific Societies in 2023: - Federation of the European Neuroscience Societies (FENS) - Italian Society of Neuroscience (SINS) - Brainstorming Research Assembly for Young Neuroscientists (BraYn) association - ALBA Network
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Niccolò Di Cintio, Research Fellow

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
● International collaborations:	
● Invited talks:	
● Science communication:	
● Editorial duties:	
● others	Courses: - How Does Myelin Contribute to Brain Plasticity? (webinair, Dec 7, 2023, Society for neuroscience)

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 2023

a. Summary (500 characters)

The major outputs of our group in 2023 was the demonstration that human striatal progenitor cells grafted into a rat model of Huntington's disease survive long term, self-organize, connect with the host circuits and counteract the deterioration of motor functions due to the lesion (Schellino et al., 2023). In a parallel study, in collaboration with Paola Berchialla's group (Dept of Clinical and Biological Sciences, Univ of Turin) we used an AI approach to classify healthy and diseased human astrocytes (Grimaldi*, Lorenzati* et al., 2023).

b. Background and rationale (3000 characters)

Our research develops around two main questions: (i) to what extent functional CNS circuits can be reconstructed through cell replacement approaches using pluripotent stem cell derived human neurons; (ii) how glia contributes to CNS physiopathology and can promote brain repair.

In 2023, our primary focus has been on the long-term therapeutic efficacy of human striatal grafts in rodent models of neurodegenerative disease. Here, our goal is to understand the extent to which adult damaged circuits can be reconstructed when new functional neuronal elements are introduced through xenografts. Specifically, we aim to determine the type of circuit reconstruction required to enhance impaired functions. These inquiries are made in the context of cell replacement approaches, involving the grafting of human striatal progenitors in a rat model of Huntington's Disease (HD). The reconstruction of striatal circuits presents unique challenges due to the necessity to rewire complex circuits with a well-defined topography. These circuits encompass both local and long-range connections, and their output relies on the balance between distinct inhibitory (direct pathways) and excitatory (indirect pathway) components. Previous analyses of xenografts at two months revealed that human grafted neurons undergo a degree of maturation and successfully project to typical striatal and extrastriatal targets. However, it is noteworthy that these grafts do not receive afferents from extrastriatal host neurons. We reasoned that longer times could be required for afferent connections to be established and that exposure to environmental enrichment, which is well known to stimulate neuroplasticity, could foster connectivity.

On the side of glial studies, we focused on Autosomal Dominant Leukodystrophy (ADLD), 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 astrocyte pathology in ADLD patients, we worked to pinpoint astrocytic dysfunctions contributing to oligodendrocyte pathology in this disease. With a similar angle we contributed to two other collaborative studies in the field of neurodevelopmental disorders aimed at studying the impact of pathological astrocyte-neuron cross-talk in Rett syndrome and at characterizing a novel in vivo model of microcephaly 17, respectively.
Finally, we tackled one of the major unresolved issues in astrocyte biology, i.e. the molecular and functional heterogeneity of astroglia across brain areas. Recent findings indicate that adult astrocyte proliferation supporting a degree of turnover may be part of such heterogeneity, as observed in mouse corpus callosum

astrocytes by M. Goetz group (LMU). In a collaborative work and based on our expertise on cerebellar astrocytes we explored whether this phenomenon extends to cerebellar white matter astrocytes or is exclusive to subcortical cells.

c. Objectives (1000 characters)

During 2023 we specifically aimed to:
(i) finalize the investigation on the impact of long-term maturation and enhanced experience through enriched environment on the maturation, connectivity and functional efficacy of human striatal grafts in rodent models of neurodegenerative disease. We expected to find an overall increased therapeutic effect and to nail down factors relevant to enhance graft efficacy.
(ii) getting further insight into glia contribution to CNS physiopathology and at devising analytic tools to capture and classify glial heterogeneity.

d. Results (4000 characters)

In 2023 we completed the revisions of two studies and contributed to 4 collaborative papers currently submitted to Science Advances and or under revision after a 1st round of review in Nature Neuroscience, iScience and Journal of Clinical Investigations.

In Schellino et al. (2023) we found that grafted human striatal cells survived up to 6 months after transplantation and showed morphological and neurochemical features typical of striatal human medium spiny neurons. Grafts wired in both local and long-range striatal circuits and formed domains suggestive of distinct striosome-like territories. Of note, complex motor performances were significantly improved by the grafts. Moreover, exposure to enriched environment selectively increased graft neuron differentiation, promoted host- to-graft connectivity and further enhanced task execution. These results demonstrated the long-term therapeutic potential of human striatal grafts for HD and show that an enriched environment can effectively promote the maturation and integration of human striatal neurons.

In Grimaldi*, **Lorenzati*** et al. (2023), we reported on the development of a protocol to obtain human astrocytes from iPSCs and of a supervised Machine Learning approach capable of classifying astrocyte morphologies based on specific features. We found that the intensity of the nuclear matrix protein LMNB1, nuclear area, cellular area, and soma area worked as important predictors of astrocyte shapes. This algorithm is used to distinguish diseased from healthy astrocytes in current studies.

New neurons can also be spontaneously generated by resident astrocytes in specific lesion conditions in the mouse striatum. In collaboration with Prof. F Luzzati (NICO) and in parallel to the investigation of the connectivity and cell type identity of these newly generated neurons, we contributed to defining the dynamics of striatal astrocyte neurogenic activation which is widespread, potentially including the entire population of striatal astrocytes (Fogli M, **Nato G**, Greulich P, Pinto J, Peretto P, **Buffo A**, Luzzati F. *Spatio-temporal dynamics of striatal astrocyte neurogenic activation after injury reveal widespread potential beyond known niches*, #adn8194 submitted to Science Adv).

In regard to astrocyte heterogeneity, single cell profiling and spatial transcriptomics of adult mouse astrocytes from the cerebral cortex and cerebellum (which we contributed) identified distinct molecular signatures in gray and white matter cells and a proliferative profile specific to subcortical white matter astroglia. Such pattern suggests that white matter astrocytes may be a novel source of adult-generated astrocytes, potentially contributing to the gray matter population in the cerebral cortex (Bocchi R, Thorwirth M, Simon T, Koupourtidou C, Clavreul S, Della Vecchia P, Wani G, Pilz G-A, **Buffo A**, Sirko S, Götz M, Fischer J. *Region-specific astrocyte heterogeneity in the White Matter reveals region-specific astrogenesis*. submitted to Nat Neurosci #NN-A83520).

Finally, we contributed to unveil the pathogenic role of astrocyte-neuron crosstalk in Rett syndrome models (Albizzati E, Florio E, Breccia M, Cabasino C, Pozzi D, **Boda E**, Battaglia C, De Palma C, Landsberger N, Frasca A. *Mecp2 KO astrocytes affect synaptogenesis by IL-6 dependent mechanisms*. Re-submitted to iScience ID: #ISCIENCE-D-23-00842), and to characterize the glial cell phenotype of a novel model of microcephaly 17 (Pallavicini G, Moccia A, Iegiani G, **Parolisi R**, Peirent ER, Berto GE, **Lorenzati M**, Tshuva RY, Balzac F, Turco E, Salvi S, Myklebust HF, Wang S, Chitale M, Girgla N, **Boda E**, Reiner O, **Buffo A**, Di Cunto F, Bielas SL. *Modelling primary microcephaly with human brain organoids reveals fundamental roles of CIT kinase activity.* Under revision J Clin Invest, ID: #175435-JCI-RG-1).

e. dvancement in the field (1000 characters)

Among published papers, we foresee that the following findings will have major impact:

- Schellino et al 2023 has already been cited in two papers, suggesting that the published findings have relevant innovative and reference content in the relevant community. Specifically, we provided the first demonstration of long term survival of striatal human grafts and that enriched environment can modulate the physiology of human neurons.
- In regard to the published algorithm for astrocyte classification, we have successfully employed that to classify healthy and diseased astrocytes in studies on human leukodystrophy.

f. Publications

1. Schellino R, Besusso D, **Parolisi R, Gómez-González GB**, Dallere S, Scaramuzza L, **Ribodino M**, Campus I, Conforti P, Parmar M, Boido M, Cattaneo E, **Buffo A**.(2023) hESC-derived striatal progenitors grafted into a Huntington's disease rat model support long-term functional motor recovery by differentiating, self-organizing and connecting into the lesioned striatum. Stem Cell Research and Therapy. 14:189.
Research article - Q1

2. Grimaldi P*, **Lorenzati* M**, **Ribodino M**, Signorino E, **Buffo A#**, Berchialla P# (2023) Predicting Astrocytic Nuclear Morphology with Machine Learning: A Tree Ensemble Classifier Study. Applied Sciences 13: 4289. * co-first auth; # co-last auth.
Research Article - Q1

3. Pallavicini G, Iegiani G, **Parolisi R**, Ferraro A, Garello F, Bitonto V, Terreno E, Gai M, Di Cunto F (2023) Lestaurtinib inhibits Citron kinase activity and medulloblastoma growth through induction of DNA damage, apoptosis and cytokinesis failure. Frontiers in Oncology. 13:1202585.
Research Article - Q2

4. Cendelin J, **Buffo A**, Hirai H, Magrassi L, Manto M, Mitoma H, Sherrard R. in Experimental Neurotransplantation for Cerebellar Ataxias. Contemporary Clinical Neuroscience, 2023, Springer Ed., Part F6, pp. 469–498. Book Chapter.

5. Martínez-Mendoza ML, Rodríguez-Arzate CA, **Gómez-González GB**, Rosas-Arellano A, Martínez-Torres A. Morphological characteristics of astrocytes of the fastigial nucleus. Heliyon. 2023 Jul 6;9(7):e18006. doi: 10.1016/j.heliyon.2023.e18006. PMID: 37483700; PMCID: PMC10362242.

6. Martínez-Mendoza ML, Rodriguez-Arzate A, **Gómez-González GB**, Kirchhoff F, Martínez-Torres A. Analysis of a cell niche with proliferative potential at the roof of the aqueduct of Sylvius. Neurosci Res. 2023 Mar; 188:28-38. doi: 10.1016/j.neures.2022.11.004.

5. Future directions and objectives for next years

a. Summary (up to 2000 characters):

In 2024 we will advance our efforts in the reconstructions of CNS circuits through cell replacement and our understanding of glia physiopathology as described below.
In regenerative medicine studies, we will start elucidating the wiring capability, molecular profile and the activity of striatal human neurons grafted in rodents and obtained with a new protocol showing increased and faster maturation.
The exploration of astrocyte heterogeneity will progress by completing the definition and validation of newly identified cerebellar astrocyte subtypes, initially discovered through sn/sc RNAseq in the postnatal and adult mouse cerebellum, with expansion to the human cerebellum. Concurrently, investigations into astrocyte pathological impact will provide insights into how dysfunctions in human iPSC-derived astrocytes promote oligodendroglia pathology in Autosomal Dominant Leukodystrophy with Autonomic Disease (ADLD). Additionally, we will assess whether these detrimental effects can be mitigated through an Allele-Specific Silencing (ASP-siRNA) strategy.
In parallel, in order to advance our understanding of oligodendroglia physiopathology, we will investigate the impact on myelin formation, maintenance and regeneration of oligodendroglial signaling molecules, environmental pollutants, and therapeutic strategies based on brain stimulation or on pharmacological approaches. In detail, we will investigate the role of anosmin1 in the axo-myelinic arrangement of nodes and initial axon segments. Moreover, we will clarify the impact of the exposure to environmental pollutants (i.e. particulate matter) on the disease course of a mouse model of Multiple Sclerosis (MS). Finally, we will study the therapeutic potential of non-invasive brain stimulation in a mouse model of myelin injury as wells as the outcome of pharmacological approaches targeting oxidative stress in instances of developmental hypomyelination.

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

Reconstruction of functional CNS circuits through cell replacement
Human neurons grafted in animal models of Parkinson's disease have shown the ability to correctly wire the host brain, marking significant progress towards the clinics. Yet, this instance includes diffuse projection systems, without defined topography. When it comes to the reconstruction of more complex circuits with a defined topography and neurochemical organization, information is scarce. Moreover, when behavioral improvement is observed, the actual mechanisms underlying such improvement remain unclear. While we have demonstrated the long-term survival and partial maturation of human striatal grafts, the obtained proportion of fully mature striatal neurons is modest. The enduring question about the comprehensive reconstruction of the direct and indirect pathways, as well as the contribution of graft activity to the observed behavioral improvement, remains unresolved. These aspects serve as the foundation for our ongoing work.
Glia physiopathology
It is now well established that glial cells are essential players for brain functions and contributors to pathology. However, many fundamental questions about glial cell specification, function in the interplay with neurons, role in pathology and actual reparative potential are still poorly understood. Moreover, the pathogenesis of diseases directly affecting glial cells remains by large unknown and effective therapies in this field are lacking.
Astrocytes (AS) comprise extremely heterogeneous types. We have been studying cerebellar AS in mouse as exemplar and unveiled fundamental cellular mechanisms implicated in the generation of their diversity (Cerrato et al., 2018; Kantzer et al., 2021). We now focus on the molecular control of AS specification and function, which is key for understanding cerebellar physiology and developing effective therapies in cerebellar diseases. We found molecular diversity across and within main AS adult types (Cerrato, unpublished observations). This extended the canonical classification and pointed to functionally specialized AS subtypes. Similar analyses in the postnatal mouse cerebellum elucidated the development

of AS types and subtypes, uncovering intrinsic and extrinsic mechanisms of cell fate determination and identifying molecularly distinct progenitor pools. Validation studies are ongoing to build a solid picture on the molecular determinants of AS type specification and their role in the cerebellum. Also, we lack information on the human cerebellum.
In demyelinating pathologies such as MS, myelin repair is inefficient, which is rooted in the dysregulation of oligodendroglia biology. Additionally, we recently showed that environmental factors, such as exposure to pollutants, negatively affect the regenerative ability of myelin (Parolisi et al., 2021). The identification of the mechanisms/factors impacting on oligodendroglia biology and myelin degeneration or regeneration is a hot topic, as it may provide targets to design preventive/therapeutic interventions. Moreover, oligodendrocytes are also affected in a number of genetic disorders associated with de- or hypo-myelination, such as ADLD and developmental diseases including the MCPH17 microcephaly. Yet, the mechanistic aspects of these pathologies are largely unknown and therapies are lacking. Specifically, although oligodendrocytes are considered as the main target in ADLD, data point also to astrocyte dysfunction. The contribution of an altered astrocyte-oligodendrocyte crosstalk in ADLD pathogenesis as well as whether this can be targeted for therapeutic purposes are still open issues. As regards MCPH17, in the Cit-k KO model the disease we found prominent alterations of oligodendroglia and other glial types which are associated with oxidative stress (Boda et al., 2022, unpublished observations). We propose to study if an early developmental antioxidant treatment can modify glial pathology and the disease trajectory.

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

In 2024, we will work toward these main general aims:
- in the study of adult circuit rewiring and cell replacement approaches, we will be focused on the reconstruction of the direct and indirect striatal pathways, as well as on the contribution of graft activity to the behavioral improvement;
- in the study of glial cells, we plan to compile an atlas of cerebellar astrocytes, clarify the pathogenetic role of ADLD astrocytes and investigate treatments mitigating and/or ameliorating glial pathology in myelin pathologies and neurodevelopmental diseases.

In detail we contribute to the mission of NICO by: (i) studying the plasticity of the injured brain and investigating cell replacement approaches for neurodegenerative pathologies; (ii) expanding the knowledge on fundamental processes of glial cell physiopathology; (ii) exploring preclinical therapeutic approaches for diseases such as Leukodystrophies, MS and microcephaly; (iii) developing innovative experimental models derived from hiPSCs.

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

Reconstruction of functional CNS circuits through cell replacement

In 2024 we will be focused on testing human striatal neurons obtained with an optimized protocol by E. Cattaneo's group (Univ Milan). This protocol increased the yield of human medium spiny neurons and accelerated their maturation in vitro (Conforti et al. 2022). We expect increased maturity and wiring capacity of these cells upon grafting, which will be investigated by conventional neurochemical analyses, single cell molecular profiling and by tracing approaches aimed to dissect the extent of actual reconstruction of the direct and indirect striatal pathways. In parallel, in both xenografts and allografts, we will examine the activity pattern of the grafted cells as well as the role of the graft activity in the functional improvements associated with the transplants. Coll: Prof E Cattaneo, Univ Milano.

Glia physiopathology

Study of astrocyte heterogeneity

We will incorporate recently published and spatial transcriptomics data into our current sc/snRNAseq datasets. This integration will enhance the resolution of our analyses, enabling the identification of functionally specialized astrocyte subtypes in situ. Additionally, we will analyze and map the morphological diversity both across and within each astrocyte type, with the goal of explore possible correlation with the cell molecular heterogeneity. In light of newly identified mature astrocyte subtypes from our sc/snRNAseq analyses, we will also finalize the study of the ontogenesis of astrocyte

heterogeneity in mice and perform confirmatory lineage tracing experiments in vivo. Analysis of sc/snRNAseq data will be integrated with multilevel validations and extended to human samples. We also plan to transfer our investigation on cerebellar AS specification on 3D cerebellar models. Coll: Prof L Telley, Univ Lausanne. Prof. M. Götz, LMU.

Understanding astrocyte and oligodendrocyte physiopathology

Transcriptional analyses revealed gene expression alterations in ADLD astrocytes. On these bases, in 2024 we will address the impact of ADLD astrocytes and of their conditioned medium on mouse and human iPSC-derived oligodendrocytes and ex-vivo myelination in organotypic cultures. Moreover, we will test whether a ASP-siRNA strategy aimed at correcting the mutation linked to ADLD, will rescue the altered phenotype and the expected detrimental effects of diseased astrocytes. Coll: Prof. E Giorgio, Univ Pavia; Prof. P Cortelli, Univ of Bologna; Prof. JG Léon, Univ of Malaga.

To investigate myelin formation, we will focus on Anosmin 1 and study the effects of its overexpression in the axo-myelinic arrangement and conduction velocity of cerebellar Purkinje cell axons. We will also screen for Anosmin 1 interactors through proteomics. Coll: Prof. F. De Castro, Cajal Institute, Madrid.

Promoting myelin repair and limiting damage in demyelinating or neurodevelopmental disorders

Enhancing neuronal activity has been shown to promote oligodendrogenesis and remyelination in preclinical studies. Thus, in 2024, we will investigate the suitability of a non-invasive brain stimulation technique (i.e. transcranial direct current stimulation - tDCS) as a therapeutic option in a mouse model of myelin injury. Collaboration with Dr. Marco Cambiaghi, Univ of Verona

Exposure to particulate matter (PM) is one of the environmental factors proposed to worsen the disease course of MS. In 2024, we will finalize a study aimed at understanding the effects - and the underlying mechanisms - of PM exposure in an mouse model of MS, i.e. Experimental Autoimmune Encephalomyelitis. Collab. with Dr. F. Montarolo, NICO and Prof. S. de Francia, UNITO.

Finally, we will investigate if and to what extent NAC (an FDA approved glutathione precursor) administration starting during either embryonic and postnatal development can revert neuroanatomical and functional traits of MCPH17 mouse mutants. Collab with Prof. A. Pistocchi (Univ of Milan) and Prof. F. Di Cunto (NICO).

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

Our research is unique in several ways:

- we leverage the originality of the angle on glia cells, which is unique in our research context
- we harness the potency of human cell models to enhance the depth of our investigations
- we employ a combination of multilevel methodological approaches and of cutting-edge techniques (see also below), which confer novelty and power to our studies
- we ask fundamental, unanswered research question by using original models and approaches:

A first 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 insights to the related fields.

We also aim to yield significant insights into glial cell specification, their intricate interplay with neurons in both physiological and pathological contexts, and their reparative potentials. These aspects are particularly crucial for gaining a complete understanding of brain development, function and dysfunction. We tackle these topics by investigating the emergence of AS heterogeneity and of the molecular substrates of oligodendrocyte biology in health and disease. In this context, the opportunity to explore the developing human cerebellum through single cell transcriptomics makes our research at the forefront of unraveling the complexity of its development. The innovative insights acquired will lay the foundation for future application, such as the generation of cerebellar organoids able to reproduce the full repertoire of neuronal and glial cells of this region that can be applied as innovative models to study cerebellar diseases.

In studies on MS - one of the major neurological disorders in Europe – we will explore the therapeutic potential tDCS. By doing so, we not only investigate a relatively unexplored avenue, but also address a significant gap in the field. Currently, there are no available therapies capable of promoting myelin restoration, thereby supporting the recovery of lost functions for MS patients who have achieved disease stabilization or who have evolved/started as progressive MS. Our research can help with answering to this unmet medical need.

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

We have strategically invested in integrating state-of-the-art technologies and advanced analytical tools to elevate the quality and multilevel comprehensiveness of our research, hence promoting the development of a robust research environment. We have been among the few laboratories at our University which first implemented these techniques.

Our recent initiatives include:

(i) developing pluripotent stem cell-based technologies as models to investigate pathological mechanisms and formulate therapies (refer to our studies on ADLD and regenerative medicine approaches);

(ii) utilizing and advancing single-cell transcriptomics and spatial transcriptomics approaches (refer to studies on AS heterogeneity). In this context, we implemented and familiarized with an innovative sc/snRNAseq technology known as SPLiT-seq. This groundbreaking method eliminates the need for specialized instrumentation, potentially bringing our institute closer to such experiments and analyses and opening the door for application in various projects across different research groups;

(iii) creating novel tools and implementing analytic 3D pipelines for retrograde, neuron-type-specific, and monosynaptic tracing of connections in the rodent brain (in regenerative medicine studies);

(iv) fine-tuning grafting technologies to distinct pathological conditions (in studies on microcephaly models and regenerative medicine);

(v) implementing technologies, including calcium photometry in vivo, optogenetics and tDCS to monitor and manipulate circuit activity in the brain (in regenerative medicine studies);

(vi) Exploring new analytical algorithms for 1) cell segmentation and transcript assignment in imaging-based spatial transcriptomics; 2) integration and label transfer between sc/snRNA sequencing and spatial transcriptomics data.

These approaches are complemented by our solid expertise in histological and high-resolution immunohistochemical analyses, behavioral studies, and mouse genomics technologies.

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2023

Laboratory name: Embryonic neurogenesis

1. LABORATORY DESCRIPTION – PERSONNEL:

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

Personnel
Pallavicini, Gianmarco, Post-docotoral 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-docotoral 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, Molecular biology and biochemical analysis 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.

Papa, Leonardo, Research Fellow, MSc in pharmaceutical chemistry and technology, 15/04/1998, +39 327 861 9230, papa.leonardo@outlook.com, Generation of new chemical inhibitors of CITK protein (joint project with IFOM, Milan).

2. CURRENT GRANTS

Starting-end date	Project Title and ID	Beneficiary	Funding Program/Agency	Role of the unit	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	UNITO
01/04/2022 31/03/2024.	Study of primary microcephaly genes as therapeutic targets for glioblastoma multiforme”	Gianmarco Pallavicini	AIRC fellowship	Coordinator	50000	UNITO
28/09/2023-27/09/2025	Dissection of common	PI	PRIN 2022 funded by EU	Coordinator	72289	UNITO

	mechanisms in genetic primary microcephaly. 2022M75NN8		through Next Generation EU program.			

3. SCIENTIFIC ACTIVITIES IN 2023

Ferdinando Di Cunto, PI

Supervised PhD students:	Direct supervisor of: Giorgia Iegiani, Giada Onorato, Alessia Ferraro. Co-supervisor of: Gianna Pavarino, Vanessa Chiappini.
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	- Prof. Stephanie Bielas, Department of Human Genetics, University of Michigan Medical School, Ann Arbor, Michigan, USA. - Prof. Shaun Stauffer, Center for Therapeutics Discovery, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio 44195, United States.
• Invited talks:	
• Science communication:	30-03-2023: BIEVOL Association (www.bievol.org) - XV BIOETHICS WEEK - Speech entitled: From pandemic to endemic: evolution of viruses and adaptation of hosts
• Editorial duties:	- Associated Editor of PLoS ONE - Associated Editor of Frontiers in Neurogenesis - Editor of a research topic for Frontiers in Neurosocience: Neurobiological underpinnings of neurodegenerative and neuropsychiatric disorders: from models to therapy (https://www.frontiersin.org/research-topics/59016/)
• others	
Organizational activities and responsibilities at NICO:	Data management
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	President of the Organizing Committee of the XX meeting of SINS (Società Italiana di Neuroscienze) - SEPTEMBER 14th – 17th, 2023 (https://sinsmeeting.com/)
Technology transfer achievements (patents, etc.):	

Gianmarco Pallavicini, Post-doctoral fellow

Supervised PhD students:	Co-supervision of Giorgia Iegiani and Alessia Ferraro
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	
• Invited talks:	

• Science communication:	
• Editorial duties:	
• others	- XX SINS meeting, Turin, Italy, 14-17 September 2023 Poster presentation: "Patients derived organoids show differences compared to mice model in DNA damage accumulations and mitotic defects leading to microcephaly syndrome"
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Christian Pritz, Post-doctoral fellow

Supervised PhD students:	
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	
• Invited talks:	
• Science communication:	
• Editorial duties:	
• others	24th InternationalC. ElegansConference, Glasgow (UK), 24-28 June 2023. Poster presentation: “Principles for coding associative memories in a compact neural network” 2nd Meeting of the ItalianC. Elegans Research Community (M.I.C.e.R.Co.) Naples Italy, 2-3 March 2023 Poster presentation: “Procedurally generated synthetic data for deep-learning-mediated semantic segmentation of micrographs”
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Giorgia Iegiani, PhD student

Supervised PhD students:	
Honors, prizes, awards:	
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.
• Invited talks:	- XX SINS meeting, Turin, Italy, 14-17 September 2023 "Citron Kinase is required for BRCA1 loading at DNA damage foci in mammalian neural stem cells and brain organoids"
• Science communication:	
• Editorial duties:	
• others	- EACR Annual Congress of the European Association for Cancer Research in Turin – Italy, 12-15 June 2023. Poster presentation: "CITK loss leads to DNA damage accumulation impairing homologous recombination dynamics and BRCA1 recruitment in medulloblastoma" - PhD meeting at Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan – Italy, 29-30 June 2023. Poster presentation: "Citron Kinase is required for BRCA1 loading at DNA damage foci in mammalian neural stem cells and brain organoids"
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Other organizational activities:	President of the National Italian Biotechnologists Association (ANBI)
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Alessia Ferraro, PhD student

Supervised PhD students:	
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	
• Invited talks:	- XX SINS meeting, Turin, Italy, 14-17 September 2023 “CITK catalytic activity inhibition through Lestaurtinb leads to DNA damage, cytokinesis failure and cell death in brain tumors.”
• Science communication:	
• Editorial duties:	
• others	- EACR Annual Congress of the European Association for Cancer Research in Turin – Italy, 12-15 June 2023 Poster presentation: “CITK catalytic activity inhibition through Lestaurtinb leads to DNA damage, cytokinesis failure and cell death in brain tumors.”
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Other organizational activities:	

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

Giada Onorato, PhD student

Supervised PhD students:	
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	
• Invited talks:	-XI Meeting Neapolitan Brain Group, 30 November 2023, Naples, Italy. Selected for oral presentation: "spaCe: assessing relative biological effectiveness of simulated deep space radiation in altering neural function in C. elegans" - 4th Memorial Maria Ciaramella, 16-17 November 2023, Naples, Italy. Selected for oral presentation: "spaCe: assessing relative biological effectiveness of simulated deep space radiation in altering neural function and survival in C. elegans" - 2nd Meeting of the Italian C. elegans Research Community (M.i.C.e.r.c.o.), 2-3 March 2023, Naples, Italy. Selected for oral presentation: "spaCe: assessing relative biological effectiveness of simulated deep space radiation in altering neural function and survival in C. elegans"
• Science communication:	
• Editorial duties:	
• others	-XX National Congress of the Italian Society for Neuroscience, 14-17 September 2023, Turin, Italy Poster presentation: "spaCe: assessing relative biological effectiveness of simulated deep space radiation in altering neural function and survival in C. elegans" - 24th International C. elegans Conference, 24-28 June 2023, Glagow, Scotland. Poster presentation: "Sex-specificity of neurodegeneration in C. elegans: the dual role of dafachronic acid"
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 2023

a. Summary (500 characters)

We study the genetic and molecular mechanisms that control neuron generation, survival and differentiation during normal brain development and how the alteration of these processes may lead to neurodevelopmental disorders, in particular primary microcephaly. To this aim, we currently use a combination of experimental and computational methods to analyze in vitro (including brain organoids) and in vivo (mouse and C. elegans) models. We also study the relevance of these mechanisms for brain cancers.

b. Background and rationale (3000 characters)

The human brain is composed of approximately 90 billion neurons, which are generated during embryonic life starting from many different types of neural stem cells, whose proliferation is extremely well organized in space and time. If too few neurons are produced, or too many neurons die during development, the brain volume can be very compromised, a condition commonly known as microcephaly. Although a significantly reduced brain volume can be compatible with normal brain function and intelligence, microcephaly is frequently associated with strongly invalidating symptoms, such as intellectual disability, epilepsy and cerebral palsy. Microcephaly can be the result of rare genetic disorders, mostly characterized by autosomal recessive inheritance. Even more frequently, it is produced by environmental factors, such as hypoxia, drugs and alcohol exposure or infectious agents, such as Rubella, Toxoplasmosis, Cytomegalovirus or Zyka virus. Research conducted in the last decade has shown that all these conditions may affect common molecular pathways, regulating genome stability, cell proliferation, cell survival and determination of cell identity.
The main focus of our group is to understand the molecular events activated by genetic and non-genetic conditions leading to normal neuronal numbers and neuronal differentiation. In particular, on the genetic side, we have been studying for many years the neurological syndrome produced in mammals by CITK inactivation, characterized by microcephaly, ataxia and epilepsy. This syndrome has recently been identified in humans with the name of MCPH17. Neural progenitors of humans or mice carrying CITK mutations fail to divide and undergo programmed cell death, leading to strong reduction of the final neuron number. During the last few years we have dedicated much effort to clarify the causal relationship between these events and the other mechanisms classically associated with microcephaly, such as asymmetric cell division of neural precursors and DNA damage. 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.

c. Objectives (1000 characters)

Specifically, our constant research aims during have so far been to clarify:
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.
5. During the last two years we have started to approach other neurodevelopmental disorders, using the power of the genetically tractable organism C. elegans. In particular, we have established a collaboration with the group of Prof. Alfredo Brusco, who has recently bcome a Faculty member of the Department of

Neuroscience, to address the biological role of mutations recently recognized as causative alterations of Autism Spectrum Disorder syndromes.

d. Results (4000 characters)

1. We have discovered that, besides impairing cytokinesis, CITK may lead to microcephaly by two additional mechanisms. The first is through an alteration of the cell division plan, which may affect cell the rate of exit from the cell cycle and therefore reduce the number of neurons possibly generated by neural progenitors. An interaction between CITK and the prominent microcephaly protein ASPM is essential for this function. The second mechanism is by directly regulating genomic stability, independently from its role in cytokinesis. Indeed, we found that cells lacking CITK display increased DNA damage early in the cell cycle. An alteration of DNA repair mechanisms may be the leading cause of this phenotype. Increased DNA damage leads to P53 activation, which is the main cause of apoptosis in CITK null models. Indeed, we found that the inactivation of P53 in CITK knockout animals leads to a disappearance of apoptosis, and strongly improves the overall neurological phenotype. The latter discovery could pave the way to the identification of new possible therapeutic strategies for apoptosis-related microcephaly.
2. CITK was originally identified as a protein important for remodeling the actin cytoskeleton. We have discovered that it may be even more important to regulate the stability of microtubules, and that this function is crucial both for completing cytokinesis and for spindle orientation.
3. 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.
5. The recruitment of Dr. Christian Pritz allowed us to implement all the necessary setups to use the C. elegans model at NICO. Using this genetically tractable system we have started to address the molecular bases of two severe neurodevelopmental disorders whose causative mutations have recently been found by Prof. Brusco laboratory.

e. 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 tumor therapy, in particular medulloblastoma. The understanding of mechanisms which may link microtubule dynamics to the maintenance of genome integrity by homologous recombination would be a major advancement in the field. Moreover, the modeling of neurodevelopmental disorders in C. elegans isexpected to provide major contribution to the molecular and cellular understanding of disease mechanisms in a wide variety of neurodevelopmental disorders.

f. Publications

Iegiani G, Ferraro A, Pallavicini G, Di Cunto F (2023) The impact of TP53 activation and apoptosis in primary hereditary microcephaly. Frontiers in Neuroscience 17:1220010
Review – Q2

Pallavicini G, Iegiani G, Parolisi R, Ferraro A, Garello F, Bitonto V, Terreno E, Gai M, Di Cunto F (2023) Lestaurtinib inhibits Citron kinase activity and medulloblastoma growth through induction of DNA damage, apoptosis and cytokinesis failure. Frontiers in Oncology 13:1202585
Research article – Q2

Pritz C, Itskovits E, Bokman E, Ruach R, Gritsenko V, Nelken T, Menasherof M, Azulay A, Zaslaver A (2023) Principles for coding associative memories in a compact neural network. Elife 12:e74434.
Research article – Q1

5. Future directions and objectives for next years

a. Summary (up to 2000 characters):

During the next three years, we plan to continue the development of the current research lines and in particular:
1. We will continue to dissect the molecular mechanisms by which Citron kinase loss leads to microcephaly. To this regard, we plan to study the mechanisms by which CITK loss alters microtubule nucleation and stability. Moreover, we will investigate how CITK mutation or inactivation leads to DNA damage. Finally, we will address the possible causal relationships between these events and will investigate how they relate to the other genes so far involved in primary microcephaly and, possibly, in medulloblastoma progression.
2. We will continue to address the role of CITK in brain tumors and in their radiosensitivity. In particular, if the AIRC grant proposal will be funded, we plan to concentrate on the development of specific CITK inhibitors.
3. We will 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. Interestingly enough, most of the identified MCPH genes play crucial roles in cell division and genomic integrity of neural progenitor cells, making them very interesting candidates as targets for drug development in brain cancers.

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

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

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2023

Laboratory name: Neuropsychopharmacology

1. LABORATORY DESCRIPTION – PERSONNEL:

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-B), 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	Funding Program/Agency	Role of the unit	Overall Amount Funded	Managed by FCO/UNITO
2023-2024	Matrix Metalloproteinase-9 and PeriNeuronal Nets: new therapeutic targets for Fragile X Syndrome	Carola Eva	PRIN: PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE	PI	204298	UNITO

3. SCIENTIFIC ACTIVITIES IN 2023

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) -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:	n/a
• Science communication:	n/a
• Editorial duties:	n/a
• others	n/a
Organizational activities and responsibilities at NICO:	In charge for hygiene anti-smoke rules
Speakers invited:	Prof Gancheva and Prof Georgieva (Medical University of Varna, Bulgaria) (19/05/2023) Prof Shimon Ben Shaabat (Ben Gurion University, Israel) (15/06/2023)
Other organizational activities:	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	
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:	
Technology transfer achievements (patents, etc.):	

Ilaria Bertocchi, Research assistant (RTD-B)

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 -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:	'Whole brain perineuronal net and parvalbumin expression analysis in Fragile X mice' Seminar at the Achucarro Basque Center for Neuroscience (Bilbao, June 2nd 2023)
• Science communication:	'La plasticità cerebrale come chiave per il trattamento della depressione' Mind to Move 16/04/2023 'Preclinical evaluation of antiepileptic drugs in the Grin2a$^{S/S}$ murine model' Symposium at 20° SINS 15/09/2023
• 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.
• others	
Organizational activities and responsibilities at NICO:	In charge for behavioral labs I and II (animal facility)
Speakers invited:	Prof Gancheva and Prof Georgieva (Medical University of Varna, Bulgaria) (19/05/2023) Prof Shimon Ben Shaabat (Ben Gurion University, Israel) (15/06/2023)
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.):	

4. Research activity in 2023

a. Summary (500 characters)

We are investigating the effects of a ketogenic diet in a mouse model of a *GRIN2A* epileptic syndrome. A first cohort of mice carrying the GluN2A(N615S) mutation and relative controls were evaluated for

cognitive function and the presence of the epileptic phenotype after being subjected to standard or ketogenic diet (KD). They were then sacrificed to collect several organs and tissues for *ex-vivo* analysis (currently ongoing).
Meanwhile, we are continuing to carry on a project addressing 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 mutations in the NMDA subunit receptor *GRIN* genes. A large fraction of these mutations leads to 'gain of function' of the NMDAR. Patients present with a combination of symptoms that includes epilepsy, intellectual disability, behavioural and motor symptoms. Controlling seizures and attenuating behavioral deficits is a significant medical need in most patients with *GRIN*-related disorders. Unfortunately, existing *in vivo* characterization of preclinical models of GRIN mutations do not adequately reproduce the human pathology (Bertocchi et al., 2023). Recently, two knock-in mice with inserted different point mutations on the GluN2A subunit were described that show an epileptic phenotype and other symptoms akin to human *GRIN*-related disorder (Amador et al., 2020; Bertocchi et al., 2021). Accordingly, a careful analysis of human *GRIN*-related mutations and rare variants reported *GRIN2A* mutations as those most involved in epileptic disorders. One of these *GRIN2A* knock-in mice, the Grin2a$^{S/S}$ mouse, is homozygous for the GluN2A(N615S) mutation, and exhibits high sensitivity to audiogenic seizures (AGS), cognitive deficits and ADHD-like behavior, faithfully summarizing what occurs in patients harboring similar mutations.
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. Fragile X syndrome is the most common monogenic cause of inherited intellectual disability and autism with an incidence of approximately 1:4000 males and 1:8000 females. FXS results from the CGG expansion at the 5'UTR of the FMR1 gene of >200 units and consequent gene silencing. FMRP protein, which is absent in FXS patients, is involved in multiple aspects of mRNA metabolism, particularly in the brain. No cure exists for FXS, and even approaches based on very promising targets (i.e. mGluR5 and GABA receptor inhibitors) failed to show clinical benefit, showing the difficulties to translate the experimental models into the clinic. Fmr1KO mice represent a useful model to study the effects of the lack of FMRP. An impairment of perineuronal nets (PNN) formation around GABAergic parvalbumin-positive interneurons in the developing auditory cortex of Fmr1KO mice was observed and pharmacological or genetic restoration of PNNs significantly ameliorates FXS-associated hyperresponsivity to acoustic stimuli. PNNs are specialized forms of extracellular matrix (ECM), which arise in concomitance with the closure of developmental critical periods of plasticity and control synaptic connectivity and functions in the adult brain. Matrix metalloproteinases (eg MMP-9), that degrade ECM and PNNs and are overexpressed in FXS, can represent a therapeutic target for FXS.
However, little is known on FXS-dependent alterations of PNNs and their components affecting activity and plasticity of other brain areas that control cognition and emotions, i.e. hippocampus, prefrontal cortex and hypothalamus.

c. Objectives (1000 characters)

Objective 1: The main aim of the first research project is to better understand the molecular mechanisms underlying the possible benefits that a KD can have on the physiology and behavior of a mouse model of *GRIN*-related disorder. For this objective we used not only homozygous (Grin2a$^{S/S}$), but also heterozygous mice (Grin2a$^{N/S}$), which are genetically more similar to the human condition; and we also considered both sexes, due to possible sex-related differences.

Objective 2: The primary initial aim of this project was to study the distribution of PNN in the whole brain to fill the lack of information related to the topic in bibliography, and to follow the PNN formation process at different times of postnatal development in brain nuclei important for the cognitive and emotional functions of Fmr1KO mice. To this scope, male and female Fmr1KO and control mice used for the experimentation were divided into 3 groups relating to the different developmental stages (I: postnatal day (P) 20; II: P40 and III: P60), obtaining a total of 12 experimental groups.

d. Results (4000 characters)

1- In parallel to the KD project, we concluded a study to assess the therapeutic efficacy of a negative allosteric modulator of the NMDAR GluN2B subunit in counteracting AGS in homozygous Grin2a$^{S/S}$ mice. The results of this work were presented in a symposium at the last 20th SINS national congress and collected in a manuscript, which was recently accepted (with only a short final revision to be done) as a short report by the British Journal of Pharmacology.

We collected an initial body of data regarding the performance of mutated (both homozygous Grin2a$^{S/S}$ and heterozygous Grin2a$^{N/S}$ mice: N2AM2S mice) and wild-type littermates, males and females, 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 cliff avoidance reaction 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). Mice were then sacrificed between P60 and P67. During the experimental protocol, the body weight gain of the animals has been recorded twice a week.
The resulting data were analyzed manually by the experimenters and 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 *ex-vivo* molecular analysis.
Our preliminary results are encouraging in some respects and can be published in a short report, but they demonstrate that the KD regimen chosen was too rigid and hard to sustain, also due to the young age of the mice used for the experiment. This will lead us to opt for the choice of a new protocol to follow in this coming year. The *ex-vivo* analysis is currently underway and we have started with the immunohistochemical analysis of the brain, while, as regards the other samples, they will be analyzed by members of the laboratory of our collaborator Prof. Collino.

2- Until now, 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.
This characterization is not yet complete because in parallel, considering that the bibliography lacks systematic analyzes of PNN distribution in the different brain regions, we have developed, thanks to our collaborations with internal NICO members (Prof Luzzati), an innovative method, which allows an automated counting of PNN and PVI in the whole-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 Luzzati 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.
A second work will arise from the completion of the analysis of the material collected in the different age phases, which will elegantly report the differences between Fmr1KO and control C57BL/6 wild type mice and between sexes (we used both hemizygous male and homozygous female Fmr1KO mice), in the process

of PNN formation along development, starting from P20 up to P60, with P40 as an intermediate stage. Furthermore, we plan to publish a third important work regarding the analysis of the maternal behavior of Fmr1KO mothers, compared to wild type C57BL/6 control ones. In fact, we found significant differences, that the scientific community will have to take into consideration when planning projects involving the use of this important model of the pathology.

e. Advancement in the field (1000 characters)

Our results will provide more insights into the molecular mechanisms of neurodevelopmental pathologies, that are also rare syndromes and currently lack effective treatments: the *GRIN*-related epileptic syndromes and the fragile X syndrome (FXS).
In particular, the soon-to-be published results cited above have clinical relevance, as the compound tested by us on our mouse model is currently in use in an ongoing clinical study in several European centers, raising hope about the results that will be obtained on patients with disorders caused by GoF mutations affecting the *GRIN2A* gene, among others (EudraCT Number: 2022-000317-14).
Surprisingly, we revealed a sex-dependent difference in AGS susceptibility and in the dose-dependent rescue effect of the tested compound as well. Particularly interesting will be an in-depth study of the causes of the sex-dependent effects observed in both research lines.

f. Publications

1. **Bertocchi I**, Cambiaghi M, Hasan MT (2023). Advances toward precision therapeutics for developmental and epileptic encephalopathies. Front Neurosci. 2023 Apr 6; 17:1140679.
MiniReview – Q2, IF:4.3

2. **Bertocchi I**, Rocha-Almeida F, Romero-Barragaˊ MT, Cambiaghi M, Carretero-Guilleˊ A, Botta P, Dogbevia GK, Trevino M, Mele P, Oberto A, Larkum ME, Gruart A, Sprengel R, Delgado-Garcıa JM* and Hasan MT* (2023). Pre- and postsynaptic N-methyl-D-aspartate receptors are required for sequential printing of fear memory engrams. iScience, Volume 26, Issue 11, 17 November 2023, 108050.
Research article – Q1, IF:5.8

3. Eltokhi A, **Bertocchi I**, Rozov A, Jensen V, Borchardt T, Taylor A, Proenca CC,1 Rawlins JNP, Bannerman DM* and Sprengel R* (2023). Distinct effects of AMPAR subunit depletion on spatial memory. Volume 26, Issue 11, 17 November 2023, 108116.
Research article – Q1, IF:5.8

4. Collotta D*, **Bertocchi I***, Chiapello E, Collino M (2023). Antisense oligonucleotides: a novel frontier in pharmacological strategy. Front. Pharmacol. 14:1304342.
Research article – Q1, IF:5.6

5. **Bertocchi I**, Turroni S, Chiazza F (2023). Editorial: Nutritional modulation of central nervous system development, maintenance, plasticity, and recovery. Front Neurosci. Nov 17; 17:1332191.
Editorial

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):

1. In the near future, we plan to study the effects of the ketogenic diet on N2AM2S mice again, but this time planning an improved administration protocol, which reduces the severe side effects observed in our previous study. 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. Another goal is to validate PNN and MMP-9 as new molecular targets and to find the most effective stage for a proper therapeutic intervention. We plan to preclinically assess new treatments of FXS at different developmental stages, opening experimental opportunities impossible to achieve with clinical studies. To do this, 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. Lastly, we are working with our longtime collaborator Prof. Hasan on active and parallel projects regarding the role of NMDA receptors in learning and memory functions, and we currently have a paper under review about astrocyte-NMDAR-dependent gliotransmission. We have planned new experiments related to the same project to collect new evidence related to communication dynamics at the level of the tripartite synapse.

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

1- Despite the demonstrated utility in some forms of developmental and epileptic encephalopathies, 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 epileptic 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- PNNs are affected in several neurodegenerative and neurodevelopmental disorders. Converging evidence from ours and others' laboratories show that PNNs are critically involved in cognitive and emotional processes in rodents and that the pharmacological manipulation of PNNs rescues behavioral deficits. Interestingly, previous studies suggest that brain plasticity and behavioral deficit may be associated with either a reduction or an increase of PV and PNN expression. MMP-9 regulates the formation and organization of PNNs through cleavage of ECM. Recent studies suggested a link between PNNs, PV and MMP-9 activity in FXS. FMRP negatively regulates MMP-9 translation and thus levels of MMP-9 are elevated in FXS. Fmr1KO mice display delayed PNN formation and increased MMP-9 activity levels in the developing auditory cortex (AC), leading to impaired development and reduced inhibitory activity of PV+ cells. Genetic or pharmacological reduction of MMP-9 levels in Fmr1KO mice normalizes PV/PNN development and reverses the hyper-responsiveness of AC neurons.

Although no approved therapies exist for FXS, there are several potential medications targeting different pathways involved in FXS pathophysiology that can be beneficial in FXS 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.

3- In a previous study, we deleted the *Grin1* gene, that encodes for the obligatory subunit (GluN1) of NMDARs, in neurons of the primary motor (M1) cortex, and found that it is needed for synaptic plasticity and trace eyeblink conditioning, a prototypical model of declarative memory (Hasan et al., 2013). 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.

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

The general aim of our research is to delve into basic nervous system mechanisms and fascinating internal and external interactions that can alter its function. For this reason, our projects fit very well with the mission of the Institute, because they concern the search for new therapeutic targets and treatments for various neuropathologies and their comorbidities. Thanks to our experimental models, at the moment the research is focused in particular on neurodevelopmental disorders, but parallel activities also concern the role of neuromodulators and specific receptors in the mature nervous system.

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

1. ‘Effects of the ketogenic diet in a mouse model of *GRIN2A* syndrome’

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 belonging to 3 different genotypes [homozygous Grin2a$^{S/S}$ and heterozygous Grin2a$^{N/S}$ mice (also called N2AM2S mice) 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 intermittently for 5 weeks from P63 to P98. During the last weeks of diet, a battery of behavioral tests will be conducted as described previously. After having evaluated the efficacy of KD on cognitive development and on AGS, we will analyze the expression of marker of neuronal plasticity and of neuroinflammation by immunohistochemistry 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. ‘Matrix Metalloproteinase-9 and PeriNeuronal Nets: new therapeutic targets for Fragile X Syndrome’

Considering that our hypothesis is that PNN alterations may be crucial for FXS pathogenesis, the main objectives of the proposed project are:

a) evaluate compounds with known effects on MMP-9 on Fmr1KO mice from molecular aspects (PNNs and their components) to synaptic, cognitive and behavioral deficits;
b) screen several compounds with known and putative effects on MMP-9/ECM using an in vitro assay and human FXS fibroblasts;
c) assess efficient compounds found through the screening proposed in point 2 in Fmr1KO mice and in human FXS iPSCs-derived neurons.

We will analyze the effectiveness of the treatment with two MMP-9 inhibitory drugs, on behavioral, molecular, biochemical and physiological defects of Fmr1KO mice at different times of development. 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.

3- 'Astrocyte NMDAR activity in cortical learning'

Based on our previous study (Hasan et al., 2013) and recently submitted research (Delgado-Garcia et al., submitted), we would like to better investigate the role of astrocyte NMDARs in the modulation of synaptic activity and learning and memory processes. Localized Ca^{2+} microdomain activity can be detected in astrocytes during information processing in the brain. However, the source and functional organization of these Ca^{2+} microdomains in relationship to behavior and neuronal activity is largely unknown. We hypothesize that such microdomains could be associated with the recruitment and activity of NMDA receptors on the astrocyte membrane. 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 and, in collaboration with NICO's members, we will perform *in vivo* two-photon microscopy in these control and KO mice.

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

The spectacular ability of neuronal network to change has fueled our interest in how early-life stress and external stimuli can alter the architecture of circuits in the limbic system, increasing susceptibility to psychopathologies. Ultimately, the major goal is to translate the acquired knowledge into new therapeutic strategies to cure psychiatric diseases and neurodevelopmental disorders.

Our research is based on the use of murine models of such disorders to identify new mechanisms and molecular targets. The search for targets and treatments is a priority for the scientific community and society in general, both for the most common and impacting diseases, and for rare ones for which there is not yet an effective cure.

The strength of our laboratory is the availability of several lines of genetically modified mice managed and maintained by us. Our skills and expertise mainly concern the analysis of animal behavior following manipulations or pharmacological treatments and *ex-vivo* analysis with basic cell biology and immunohistochemical methods. Despite this, thanks to the great potential and opportunities that NICO offers us, and to our various collaborations, we have also been able to carry out experiments that use cutting-edge technologies and approaches.

Our work on memory engram using the pre- and postsynaptic NMDAR plasticity, and on interrogating the role of specific NMDAR expressed by astrocytes, provide enormous possibilities for applying the genetic technologies developed by our collaborator (and used in our studies) to reveal fundamental insights in different neuropsychiatric disorders in the future.

Thanks to our collaborators in Rome (FXS project), we can have access to cells derived from patients and carry out screening of new molecules using biochemical approaches, and thanks to our collaborators at NICO we have carried out state-of-art imaging analyzes and we can count on their help in the use of machinery that requires specific skills. Thus, I must say that the most beautiful goals and works of our research have been achieved thanks to constructive exchange and collaborations.

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 2023

Laboratory name: Neuroendocrinology

1. LABORATORY DESCRIPTION – PERSONNEL:

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: RTD-B, in maternity from august 2023, 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 UNITO Research fellow from January to August 2023, degree: PhD, birthdate: 30/01/1992, phone: 0116706632,
email: brigitta.bonaldo@unito.it,
Role & expertise: Researcher; Neurodegenerative disorders models, endocrine disruptors

3) 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

4) 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

5) Surname: Bellantoni, name: Mariateresa, position: BBRF/NICO Research fellow from January to September 2023, degree: Master Degree, birthdate: 28/12/1995, phone: 0116706632, email: mariateresa.bellantoni@edu.unito.it,
role & expertise: Researcher; endocrine disruptors

6) Surname: Ricci, name: Elena, position: BBRF/NICO Research fellow from September 2023, degree: Master Degree, birthdate: 12/06/1998, phone: 0116706632,
email: elena.ricci@edu.unito.it,
role & expertise: Researcher; endocrine disruptors

2. CURRENT GRANTS

Starting-end date	Project Title and ID	Beneficiary	Funding Program/Agency	Role of the unit	Overall Amount Funded	Managed by FCO/UNITO
2019-2023		Panzica-Gotti, PI	UNITO-autofinanziata	Coordinator	13000	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 2023

Stefano Gotti, (PI)

Supervised PhD students:	Chiara Ballan Casile Antonino (with Prof. Cifani, University of Camerino)
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:	
• Science communication:	
• Editorial duties:	Journal Reviewer: Brain Research, Journal of Chemical Neuroanatomy, Cell and Tissue Research, Physiology and Behavior, Neurological Science, Histology and Histopathology, Neurobiology of Disease, Molecular and Cellular Neuroscience
• others	
Organizational activities and responsibilities at NICO:	First aid and fire safety officer
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	Organizer of the virtual Symposium “State of the art on Steroids and Nervous System: In memory of Giancarlo”- February 2023 Organizing the 12th Meeting Steroids and Nervous System, that will be held in Torino-February 2024
Technology transfer achievements (patents, etc.):	

ALL LAB MEMBERS

Activities:	**Marraudino**. Ricercatore in classe. Dissemination meetings for 'Researcher in the classroom' project of the Umberto Veronesi Foundation aimed at high schools: • 17/03/2023, IIS Aldo Moro, Rivarolo Canavese (TO). • 20/04/2023, IIS Primo Levi, Torino. • 21/04/2023, Liceo Linguistico ‘Albert Einstein’, Torino.

4. Research activity in 2023

a. Summary (500 characters)

In 2023 we mainly focused our activity in two topics: a) studies related on Endocrine Disruptors: in particular, we studied the effect of Endocrine Disruptors exposure in different nervous circuits

and related behaviors; b) studies related on Gaming Disorders: our efforts were aimed to validate a rat animal model for studying the Gaming Disorder develop at NICO.

b. Background and rationale (3000 characters)

In the Nervous System, many biological effects are mediated by steroid hormones: 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 of our research lines 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 of our lab involves the creation of a rat model of gaming disorder (GD). 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 are interested in sex differences in addictive behavior and brain activity during play and in possible alteration in several circuits involved in reward system. We developed and validated a rat animal model for studying the Gaming Disorder (***Casile et al., submitted to Psychopharmacology, in revision***).

c. Objectives (1000 characters)

Our main goal regarded the study of EDCs; in particular, the study of the interactions among EDCs and neural circuits-behavior. We analyzed the effect of perinatal exposure to different EDCs. We focalized our attention to neuroendocrine circuits controlling feeding behavior, and anxiety behavior.

d. Results (4000 characters)

Perinatal exposure to Endocrine Disruptors Compounds

Exposure to EDCs is especially dangerous if it occurs during specific “critical periods” of life, when organisms are more sensitive to hormonal changes. EDCs may influence different aspect of life and the maternal behavior.

We studied (**1**) the effects of perinatal exposure to Bisphenol A (BPA) and of its substitute Bisphenol S (BPS) administered in low doses throughout pregnancy and lactation.

In particular, we administered adult C57BL/6 J females mice orally with BPA, BPS, or vehicle from mating to offspring weaning. We assessed the number of pups at birth, the sex ratio, and the percentage of dead pups in each litter; during the first postnatal week we observed spontaneous

maternal behavior. At the weaning of the pups, we sacrificed the dams and analyzed the oxytocin system, known to be involved in the control of maternal care, in the hypothalamic magnocellular nuclei. At birth, pups from BPA-treated dams tended to have a lower male-to-female ratio compared to controls, while the opposite was observed among BPS-treated dams' litters. During the first postnatal week, offspring mortality impacted differentially in the 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-treated dams spent significantly less time in pup-related behaviors than controls. Oxytocin immunoreactivity in the paraventricular and supraoptic nuclei was increased only in the BPA-treated dams. Exposure to BPs during sensitive developmental periods represents a risk for both dams and offspring, even at low environmentally relevant doses, through the functional alteration of neural circuits controlling fundamental behaviors for pup survival, such as maternal behaviors.

Another study (**2**) is focused on the exposure to Organotins such as tributyltin chloride (TBT), a highly diffused environmental pollutants, which act as metabolism disrupting chemicals, interfering with fat tissue differentiation, and impairing the control of energetic balance.
We orally administered daily a solution containing oil, or TBT in different concentrations (0.25, 2.5, or 25 μg/kg body weight/day) to pregnant females 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 at the level of the paraventricular nucleus, with a decrease in immunoreactivity in both sexes (significant in females for all TBT doses and in males only for intermediate TBT doses), while no effect was observed in other hypothalamic areas. Metabolic syndrome, as well as obesity and diabetes, which are significant health issues, are considered multifactorial diseases and may be caused by exposure to metabolic disruptors, both in adults and during perinatal life. Our work indicates that TBT doses defined as the tolerably daily intake had a profound and sex-specific long-term effect.

Epidemiological studies support the idea that multiple sclerosis (MS) is a multifactorial disease, overlapping genetic, epigenetic, and environmental factors. A better definition of environmental risks is critical to understand both etiology and the sex-related differences of MS. Exposure to EDCs fully represents one of these risks. Among synthetic EDCs, exposure to BPA has been implicated in the etiology of MS, but to date, controversial data has emerged. Furthermore, nothing is known about BPS, one of the most widely used substitutes for BPA. As exposure to bisphenols will not disappear soon, it is necessary to clarify their role also in this pathological condition defining their role in disease onset and course in both sexes. In this study (**3**), we examined, in both sexes, the effects of perinatal exposure to BPA and BPS in one of the most widely used mouse models of MS, experimental autoimmune encephalomyelitis (EAE). Exposure to bisphenols seemed to be particularly deleterious in males. In fact, both BPA- and BPS-treated males showed anticipation of the disease onset and an increased motoneuron loss in the spinal cord. Overall, BPA-treated males also displayed an exacerbation of EAE course and an increase in inflammation markers in the spinal cord. Analyzing the consequences of bisphenol exposure on EAE will help to better understand the role of both xenoestrogens and endogenous estrogens on the sexually dimorphic characteristics of MS.

Focusing on the perinatal exposure to BPA or BPS, we investigated (**4**) the effects on anxiety-related behaviors and the serotonergic system, which is highly involved in controlling these behaviors, in adult mice. We treated C57BL/6J mice dams orally with a dose of 4 μg/kg body weight/day (i.e., EFSA TDI) of BPA or BPS, at the onset of mating and continued treatment until the offspring were weaned. Adult offspring of both sexes performed the elevated plus maze and the

open field tests. Then, we analyzed the serotonergic system in dorsal (DR) and median (MnR) raphe nuclei by immunohistochemical techniques. Behavioral tests highlighted alterations in BPA- and BPS-treated mice, suggesting different effects of the bisphenols exposure on anxiety-related behavior in males (anxiolytic) and females (anxiogenic). The analysis of the serotonergic system highlighted a sex dimorphism in the DR only, with control females showing higher values of serotonin immunoreactivity (5-HT-ir) than control males. BPA-treated males displayed a significant increase of 5-HT-ir in all analyzed nuclei, whereas BPS-treated males showed an increase in ventral DR only. In females, both bisphenols-treated groups showed a significant increase of 5-HT-ir in dorsal DR compared to the controls, and BPA-treated females also showed a significant increase in MnR. These results provide evidence that exposure during the early phases of life to BPA or BPS alters anxiety and the raphe serotonergic neurons in a sex-dependent manner.

e. Advancement in the field (1000 characters)

Our recent 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 and modification of some related behaviors. More important, we have also shown that one of the main substitutes for BPA, BPS, causes harmful effects if not worse than the BPA.

f. Publications

1) Bonaldo B, Gioiosa L, Panzica G, Marraudino M. (2023) Exposure to either Bisphenol A or S represents a risk for crucial behaviors for pup survival, such as spontaneous maternal behavior in mice. Neuroendocrinology; 113(12): 1283-1297.
Research article – Q1

2) Ponti G, Bo E, Bonaldo B, Farinetti A, Marraudino M, Panzica G, Gotti S. (2023) Perinatal exposure to tributyltin affects feeding behavior and expression of hypothalamic neuropeptide Y in the paraventricular nucleus of adult mice. J Anat. Feb; 242(2): 235-244.
Research article – Q1

3) Bonaldo B, Casile A, Montarolo F, Bettarelli M, Napoli F, Gotti S, Panzica G, Marraudino M. (2023) Effects of perinatal exposure to bisphenol A or S in EAE model of multiple sclerosis. Cell Tissue Res. May; 392(2): 467-480.
Research article – Q1

4) Bonaldo B, Casile A, Ostuni MT, Bettarelli M, Nasini S, Marraudino M, Panzica G, Gotti S. (2023) Perinatal exposure to bisphenol A or S: Effects on anxiety-related behaviors and serotonergic system. Chemosphere. Nov 30; 349: 140827.
Research article – Q1

Collaborations among NICO groups:
Bonaldo B, Casile A, Montarolo F, Bertolotto A (2023) Modeling Multiple Sclerosis in the Two Sexes: MOG35-55-Induced Experimental Autoimmune Encephalomyelitis. J Vis Exp. 2023 Oct 13;(200).
Research article – Q2

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. 2023 Jan 18;11(1):16
Research article – Q1

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 kisspeptin hypothalamic circuits, and sexual-related behaviors. The kisspeptin system is highly involved in the control of key aspects of reproductive functions, such as regulation of puberty onset and estrous cycle, and peculiar behaviors like mate-preference and lordosis. The kisspeptin system is also known to be a target of endocrine disruption although few study directly investigated the effects of bisphenols.

2 - Effects of Genistein (and gonadal hormones antagonists) in neural circuits controlling reproduction, metabolism, and other physiological parameters. Genistein, 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.

3 - Effects of drugs treatment in a rodent model of Gaming Disorder. After developing and validating the gaming disorder model we want to try to use some drugs tested for "addiction" treatment on the model itself to see the consequences.

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

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

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 collectively named Endocrine Disrupting Chemicals (EDCs) may alter the functions of the endocrine system which is intimately connected to the development and functioning of the nervous system. Many EDCs 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. 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 our research towards two main topics:

- **EDCs and steroids hormones effects**

Bisphenols effects.

we want to study the effects of Bisphenols on the hypothalamic kisspeptin system and reproductive behaviors in mice. The kisspeptin system, in fact, is highly involved in the control of reproductive functions, such as regulation of puberty onset and estrous cycle. The kisspeptin system is also a target of endocrine disruption although few study directly investigated the effects of bisphenols. We will study the effects of bisphenols exposure on this system.

GEN and 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).

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 treat 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.

- **Translational studies**

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. After the validation of our rat model (our paper related to the production of this model is currently under revision), we will study the effect of drugs used for addiction treatment in our GD model.

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 2023

Laboratory name: Peripheral Nerve Regeneration Unit

1. LABORATORY DESCRIPTION – PERSONNEL:

Principal Investigator

Raimondo Stefania (Acting Group leader as Stefano Geuna, Rector of the University of Turin)
Full Professor (from 1/12/2023)
PhD in Physiology,
Master's degree in Biological Sciences, University of Turin
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
Email: giulia.ronchi@unito.it
Role & expertise: In vivo models for peripheral nerve regeneration study

Gambarotta Giovanna
Position: Associate Professor
Degree: PhD in Cellular Sciences and Technologies,
Master's degree in Biological Sciences, University of Turin
Birthdate: 22/08/1967
Phone: +39 011/6705436
Email: giovanna.gambarotta@unito.it
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
Phone: +39 011/6705433
Email: federica.fregnan@unito.it
Role & expertise: In vitro model for peripheral nerve regeneration study

Muratori Luisa
Position: Research Assistant
Degree: PhD in Experimental Medicine and Therapy,
Master's Degree in Neurobiology, University of Turin
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

El Soury Marwa (from 01/12/2022)
Position: Post-doctoral fellowship recipient
Degree: PhD in Neuroscience,University of Turin,
Master's Degree in Molecular Biology and Biotechnology, Alexandria University
Birthdate: 22/04/1991
Phone: +39 011/6705436
Email: marwa.elsoury@unito.it
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 Turin
Birthdate: 04/08/1987
Phone: +39 011/6705433
Email: alessandro.crosio@unito.it
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
Birthdate: 06/04/1995
Phone: +39 011/6705436
Email: federica.zen@unito.it
Role & expertise: Biomolecular analysis of peripheral nerve regeneration

Garcia Bejarano Marina
Position: PhD student, PhD in Neuroscience
Degree: Master degree in Tissue Engineering and Advanced Therapies, University of Granada
Birthdate: 14/10/1996
Phone: +39 011/6705436
Email: marina.garciabejerano@unito.it
Role & expertise: Biomolecular analysis of peripheral nerve regeneration

Metafune Miriam
Position: PhD student, PhD Program in Experimental Medicine and Therapy (from 01/11/2023)
Degree: Master Degree in Molecular Biology, University of Parma
Birthdate: 18/12/1994
Phone: +39 011/6703577
Email: miriam.metafune@unito.it
Role & expertise: Study of the capability of biomaterials to promote peripheral nerve regeneration.

Pellegrino Davide
Position: PhD student, PhD Program in Experimental Medicine and Therapy (from 01/11/2023)
Degree: Master's Degree in Cellular and Molecular Biology, University of Turin
Birthdate: 28/02/1998
Phone: +39 011/6705436
Email: da.pellegrino@unito.it
Role & expertise: In vitro and in vivo models for studying the role of gut microbiota in peripheral nerve regeneration

Molinaro Debora

Position: Fellowship recipient
Degree: Master degree in Cellular and Molecular Biology, University of Turin
Birthdate: 12/11/1991
Phone: +39 011/6705436
Email: debora.molinaro@edu.unito.it; debora.molinaro.to@gmail.com
Role & expertise: In vitro and in vivo models for peripheral nerve regeneration

Bertone Francesca
Position: Fellowship recipient (from 01/12/2023)
Degree: Master degree in Medical Biotechnology, University of Turin
Birthdate:16/09/1997
Phone: +39 011/6703577
Email: francesca.bertone@icloud.com
Role & expertise: in vitro models for nervous system regeneration, biochemical analysis of nervous tissue and behavioural tests on murine models.

2. CURRENT GRANTS

Starting-end date	Project Title and ID	Beneficiary	Funding Program/Agency	Role of the unit	Overall Amount Funded	Managed by FCO/UNITO
12/2022 - 11/2026	D34Health	Stefania Raimondo	PNRR MUR/MH	Team component	325.895	UNITO
10/2023 - 09/2025	Development of nano/micro-engineered devices for applications in peripheral nervous system pathological models	Stefania Raimondo	PRIN-MUR	PI of research Uni	59.238	UNITO
12/2022 - 12/2023	Cadaveric dissection for medical training	Stefania Raimondo	Grant for Internationalization GFI - UNITO	Coordinator	22.666,67	UNITO
10/2023 - 09/2025	Gut and NeuroMuscular system: investigating the impact of microbiota on nerve regeneration and muscle reinnervation after	Giulia Ronchi	PRIN-MUR	Coordinator	106.345	UNITO

	peripheral nerve injury (Gut-NeuroMuscle) 20227YB93W					
12/2023 - 11/2025	New insights into myelin maintenance and peripheral nerve regeneration: the role of Beclin1 (INNER-BECN1) P2022Y2A3L	Giulia Ronchi	PRIN-PNRR	PI of research Uni	107.130	UNITO
12/2022 - 12/2023	Investigating the effect of microbiota on peripheral nerve regeneration	Giulia Ronchi	Grant for Internationalization GFI - UNITO	Coordinator	15.233	UNITO
12/2022 - 12/2023	Nerve conduit functionalization with nanoparticles for the controlled release of recombinant factors promoting nerve regeneration	Giovanna Gambarotta	Grant for Internationalization GFI - UNITO	Coordinator	11.000	UNITO
01/2022 - 12/2023	-HUMAn multi-tissue platform for comprehensive evaluation of chemical TOXicology on a CHIP	Giovanna Gambarotta	Ex-post di progetti di ricerca di Ateneo - Compagnia di San Paolo	Coordinator	51.560	UNITO
01/2023-12/2023	Investigating the ability of dECM hydrogel to sustain nerve regeneration in vitro and ex vivo	Luisa Muratori	Grant for Internationalization GFI - UNITO	Coordinator	15.000	UNITO

3. SCIENTIFIC ACTIVITIES IN 2023

Stefania Raimondo (PI)

Supervised PhD students:	Alessandro Crosio (PhD) Federica Zen (PhD) Monica Maurina (MD/PhD)

	Miriam Metafune (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 - Universidade de Lisboa, Portugal, Dr. André Luís Bombeiro - Kyushu Institute of Technology, Fukuoka, Japan, prof. Yuki Shirosaki
● Invited talks:	- Keynote lecture titled “Tissue engineering and Peripheral nerve regeneration” in the congress AL4Animals "Comparative and Translational Medicine and Biotechnology", 20th-21st October 2023, Porto, Portugal - Title of the lecture: “Anatomage Table for medical education”. Event: “Navigating Simulation in Medicine from Student to Professional Perspectives: The 1st Bilateral Israel-Italy Event”. Haifa, Israel, May 2023.
● Science communication:	
● Editorial duties:	- Editorial Board Member of Frontiers in Neuroanatomy - Editorial Board Member of Biomedicines - Guest Associate Editor for the special issue “Advance Research in Peripheral Nerve Regeneration" for the journal Biomedicines. - Guest Associate Editor for the special issue “Recent Advances in the Anatomy, Physiology, And Pathophysiology of the Peripheral Nervous System” for Frontiers in Neuroanatomy.
● others	- Referee for grant agencies: FWO (Fonds voor Wetenschappelijk Onderzoek - Vlaanderen) - 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:	
Speakers invited:	Prof. Elif KESKİNÖZ, University of Acibadem, Istanbul, Turkey
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Giulia Ronchi, Associate Professor

Supervised PhD students:	Monica Maurina (MD/PhD) Riccardo Aucello (PhD) Davide Pellegrino (PhD)
Honors, prizes, awards:	

Outreach activities	
● International collaborations:	- University of Hannover, Germany, prof Kirsten Haastert-Talini - Universidade de Lisboa, Portugal, Dr. André Luís Bombeiro - University of Porto, Portugal, prof. Ana Colette Maurício
● Invited talks:	
● Science communication:	
● Editorial duties:	- Guest Editor of the Special Issue "Regenerative Medicine for Peripheral Nerve Injury: Recent Advances, Emerging Therapies and Future Directions" in the International Journal of Molecular Sciences - Youth Editorial Board Member of Neural Regeneration Research
● others	Reviewer for Austrian Science Fund (FWF)
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Giovanna Gambarotta, Associate Professor

Supervised PhD students:	Marina Garcia Bejarano (PhD in Neuroscience) in cotutelle with University of Zaragoza (prof Jesus M. de la Fuente) Federica Zen (PhD) (co-supervised)
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 M. de la Fuente
● Invited talks:	- Keynote lecture at the IV Conferences in Applied Tissue Engineering, 23-05-2023 Granada, Spain, on "Cells and factors involved in the regeneration of peripheral nerves".
● Science communication:	
● Editorial duties:	- Editorial Board Member of Brain Sciences - Associate Editor for Cellular Neuropathology (section Frontiers in Cellular Neuroscience)
● others	- Project Reviewer for Austrian Science Fund (FWF)
Organizational activities and responsibilities at NICO:	
Speakers invited:	Prof. Kusakabe, Okawa and Maruo (Konan University, Japan) NICO NeuroWebinars - 12-10-2023 (HYBRID)
Other organizational activities:	

Workshops, Schools or Conferences organized:	Member of the local organizing committee of the 20th National Congress of the Italian Society for Neuroscience (SINS), 14-17/09/2023 Torino
Technology transfer achievements (patents, etc.):	Italian patent for Industrial Invention number 102015000071499 entitled "Conjugate of Neuregulin 1 for the treatment of peripheral nerve lesions", release date: 18 April 2018, expiry: 11 November 2035. Inventors: Stefano Geuna, Abraham Shahar, Ofra Ziv-Polat, Giovanna Gambarotta, Federica Fregnan. Application no. filed with the Ministry of Economic Development

Federica Fregnan, Research technician

Supervised PhD students:	Alessandro Crosio (PhD) Federica Zen (PhD) Miriam Metafune (PhD)
Honors, prizes, awards:	National Scientific qualification as associate, Academic Recruitment Field 05/H, 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;
Outreach activities	
● International collaborations:	- Kyushu Institute of Technology, Fukuoka, Japan, prof. Yuki Shirosaki - University of Hannover, Germany, prof Kirsten Haastert-Talini
● Invited talks:	
● Science communication:	Oral presentation at meetings: -Federica Fregnan, Luisa Muratori, Marwa El Soury, Federica Zen, Ilaria Tonazzini, Luca Scaccini, Francesco Porpiglia, Stefano Geuna, Stefania Raimondo "Improving regenerative capabilities of peripheral nervous cells with chitosan microstructured and functionalized membranes." 76° SIAI Congress, 11-13 September, Modena Italy. Poster presentation at meeting: -Luisa Muratori, Federica Fregnan, Alessandro Crosio, Federica Zen, Ilaria Tonazzini, Juliette Meziere, Francesco Porpiglia, Stefano Geuna, Stefania Raimondo. "Strategies to improve the regeneration of cavernous nerves after radical prostatectomy". Satellite Event of 20th National Congress Of The Italian Society For Neuroscience (SINS), Turin, September 14th – 17th , 2023".
● Editorial duties:	Guest Associate Editor for the special issue "Recent Advances in the Anatomy, Physiology, And Pathophysiology of the Peripheral Nervous System" for Frontiers in Neuroanatomy.

• others	
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	Italian patent for Industrial Invention number 102015000071499 entitled "Conjugate of Neuregulin 1 for the treatment of peripheral nerve lesions", release date: 18 April 2018, expiry: 11 November 2035. Inventors: Stefano Geuna, Abraham Shahar, Ofra Ziv-Polat, Giovanna Gambarotta, Federica Fregnan. Application no. filed with the Ministry of Economic Development

Luisa Muratori, Research Assistant

Supervised PhD students:	Alessandro Crosio (PhD) Miriam Metafune (PhD)
Honors, prizes, awards:	National Scientific qualification as associate, Academic Recruitment Field 05/H, 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;
Outreach activities	
• International collaborations:	-University of Zaragoza, Spain, prof Jesus Ciriza -Kyushu Institute of Technology, Fukuoka, Japan, prof. Yuki Shirosaki -University of Hannover, Germany, prof Kirsten Haastert-Talini
• Invited talks:	
• Science communication:	
• Editorial duties:	
• others	Oral presentation at meetings: -Luisa Muratori, Federica Fregnan, Alessandro Crosio, Federica Zen, Matteo Manfredi, Ilaria Tonazzini, Juliette Meziere, Francesco Porpiglia, Stefano Geuna, Stefania Raimondo. "Strategies to improve cavernous nerve regeneration after radical prostatectomy". 76° SIAI Congress, 11-13 September, Modena Italy. -Luisa Muratori, Arianna Lovati, Alessandro Crosio, Debora Molinaro, Stefania Raimondo. "Acellular pig nerve graft to repair median nerve injury: a preliminary study on rats". XXXIII Congresso GISN , Verona 24-25 Novembre 20203. Poster presentation at meeting: -Luisa Muratori, Federica Fregnan, Alessandro Crosio, Federica Zen, Ilaria Tonazzini, Juliette Meziere, Francesco Porpiglia, Stefano Geuna, Stefania Raimondo. "Strategies to improve the

	regeneration of cavernous nerves after radical prostatectomy". Satellite Event of 20th National Congress Of The Italian Society For Neuroscience (SINS), Turin, September 14th – 17th , 2023".
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 2023

a. Summary (500 characters)

Research activities have been focused on peripheral nerve repair and regeneration. In detail, on: 1.understanding biomolecular and biological processes occurring during nerve regeneration within different nerve conduits; 2.methodology and techniques to repair severe somatic and autonomic injury with nerve substance loss; 3.evaluation of microbiota influence on peripheral nervous system development; 4.innovative biomaterials and tissue engineering for nerve repair and in vitro model development.

b. Background and rationale (3000 characters)

Peripheral nerves often suffer from lesions due to trauma or medical interventions, leading to significant declines in motor and sensory functions, greatly affecting patients' quality of life. While the peripheral nervous system (PNS) maintains a notable capacity for regeneration even in adulthood, the recovery from injuries is typically inadequate, particularly in cases of extensive nerve damage or loss. The growing number of patients undergoing nerve surgery is a strong motivation for increased research in the area of peripheral nerve regeneration. This includes the development of innovative approaches to enhance functional recovery. In severe cases, particularly limb injuries involving substantial nerve loss, direct repair isn't feasible. Instead, grafting is necessary to connect the severed nerve ends. Nerve fibers can regenerate through these grafts, eventually reconnecting with their original peripheral targets. Although autologous sensory nerve segments are highly effective and commonly used for grafting, their use requires harvesting healthy nerves, necessitating additional surgery and leading to residual sensory deficits.

Consequently, alternative graft materials, both biological and synthetic, have been developed and are being used clinically. Nerve structure lesions can result in reduced or complete loss of sensitivity and/or motor function in the affected area. Since the clinical outcomes of nerve injuries are often unsatisfactory and full functional recovery is rare, more research in this field is crucial.

Several factors influence the outcome, including the injury location, time elapsed before surgical repair, the denervated muscle's ability to accept reinnervation and recover from atrophy, the diminished regeneration capacity of injured axons after prolonged axotomy, the loss of Schwann cell support for regeneration, patient age, and co-existing health conditions.

This research encompasses various disciplines, aiming not only to deepen our understanding of the biological processes following nerve damage but also to determine the most effective strategies for optimizing post-traumatic nerve regeneration and ultimately restoring the patient's motor and sensory functions.

c. Objectives (1000 characters)

The objectives of the group activities were to 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 development; 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)

Strategies to improve outcome after periprostatic nerve bundles injury

Radical prostatectomy for prostate cancer resections results in erectile dysfunction due to damage of the periprostatic nerve bundles.

Two surgical strategies to improve the outcome were studied:

1.In a small patients' group, the novel strategy "nerve transfer" was applied: penis dorsal nerve neurotization with femoral nerve through a sural nerve cable graft. This surgical procedure was tested on a rat animal model with a direct motor nerve transfer to corpora cavernosa with good results regarding the feasibility and safety of the surgical technique.

2.Functionalized chitosan membranes functionalized and not were applied in vivo to repair cavernous nerve injury on adult rats. The post recovery time was extended to 120 days to evaluate the different device ability to allow autonomic axonal regeneration and functional recovery.

Decellularized extracellular matrix (dECM) hydrogel to sustain nerve regeneration.

A dECM hydrogel obtained from human cadaver skin, was tested in vitro to study dECM molecules (collagen, elastin, fibronectin, laminin, keratin) ability to promote Schwann cell proliferation and migration, and to allow neurite outgrowth in neuronal cell lines and sensory neuron primary cultures.

Decellularized porcine nerves for repairing peripheral nerve injuries

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. Decellularized porcine nerves were successfully used as a graft to repair a nerve injury on rats providing a promising alternative to autograft technique.

Microbiota and peripheral nervous system

The analysis of the effect of the microbiota metabolite short-chain fatty acids on Schwann cell activity in vitro started. In particular, propionate and butyrate (but not acetate) were shown to exert a proliferative and antioxidant effect on primary Schwann cells.

Vascularization role in nerve regeneration

Conduits used to bridge nerve gaps are effective like autograft (the gold standard technique) when gaps are short. Understanding nerve regeneration within short gaps could help improve their efficacy for longer gaps. Endothelial cells were shown to form a capillary network used by Schwann cells to colonize conduits before axon regrowth. As "muscle-in-vein" is a repair technique used to efficiently repair nerve gaps, vascularization and Schwann cell migration within chitosan conduits and veins filled with muscle fibers was analysed, to investigate their contribution in vascularization, Schwann cell migration and nerve regeneration.

Innovative biomaterials to promote peripheral nerve regeneration.

An innovative Glucidex®-derived membrane was assessed for its ability to promote nerve regeneration in traumatic somatic and iatrogenic autonomic injuries. Analyses of Glucidex®

membrane dissolution products revealed a maintained Schwann cell proliferation, with organized actin cytoskeleton and lamellipodia indicating cell migration, crucial for glial support in nerve regeneration. Exploring apoptosis and cellular alterations related to Bax and Bcl-2 proteins showed no association with cell death; instead, membrane dissolution products supported good cell survival.

Development of an in vitro model using human induced pluripotent stem cells (hiPSC)

"Nerve-on-a-chip" represents a good experimental model to study peripheral nerves in vitro. To this aim, in collaboration with E. Signorino (Nico), the ability to cultivate human induced pluripotent stem cells (hiPSC) and to stimulate their differentiation towards a motor neuron phenotype was acquired.

At the same time, proliferation and vitality assays were carried out using Schwann cell and dorsal root ganglia primary cultures cultured on a 3D scaffold developed by bioengineers (Politecnico of Torino).

e. Advancement in the field (1000 characters)

The main advancements reached with our research activities during 2023 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 demonstrated a proliferative and an antioxidant effect of the microbiota metabolite short-chain fatty acids on Schwann cells in vitro. iii) We demonstrated the regeneration of the autonomic nervous system after iatrogenic damage resembling the injury of the neurovascular bundles in humans during radical prostatectomy. iv) For the first time, we studied the in vivo implantation of a decellularized porcine nerve as a xenograft model to repair a rat median nerve injury. v) New promising biomaterials have been tested.

f. Publications

1. Shirosaki Y, **Fregnan F, Muratori L**, Yasutomi S, **Geuna S, Raimondo S** (2023) The Impact of the Molecular Weight of Degradation Products with Silicon from Porous Chitosan-Siloxane Hybrids on Neuronal Cell Behavior. Polymers (Basel) 15:3272. Research article – Q1
2. **El Soury M**, García-García ÓD, Tarulli I, Chato-Astrain J, Perroteau I, **Geuna S, Raimondo S, Gambarotta G**, Carriel V (2023) Chitosan conduits enriched with fibrin-collagen hydrogel with or without adipose-derived mesenchymal stem cells for the repair of 15-mm-long sciatic nerve defect. Neural Regen Res 18:1378-1385. Research article – Q2
3. Calabrò S, Kankowski S, Cescon M, **Gambarotta G, Raimondo S**, Haastert-Talini K, **Ronchi G** (2023) Impact of Gut Microbiota on the Peripheral Nervous System in Physiological, Regenerative and Pathological Conditions. Int J Mol Sci 24:8061. Review– Q1
4. Arrigo E, Gilardi S, **Muratori L, Raimondo S**, Mancardi D (2023) Biological effects of sub-lethal doses of glyphosate and AMPA on cardiac myoblasts. Front Physiol 14:1165868. Research article – Q2
5. García-García ÓD, **El Soury M**, Campos F, Sánchez-Porras D, **Geuna S**, Alaminos M, **Gambarotta G**, Chato-Astrain J, **Raimondo S**, Carriel V (2023) Comprehensive ex vivo and in vivo preclinical evaluation of novel chemo enzymatic decellularized peripheral nerve allografts. Front Bioeng Biotechnol 11:1162684. Research article – Q1
6. **Ronchi G, Fregnan F, Muratori L, Gambarotta G, Raimondo S** (2023) Morphological Methods to Evaluate Peripheral Nerve Fiber Regeneration: A Comprehensive Review. Int J Mol Sci 24:1818. Review– Q1

7. Civra A, Costantino M, **Ronchi G**, Pontini L, Poli G, Marinozzi M, Lembo D (2023) Identification of oxysterol synthetic analogs as a novel class of late-stage inhibitors of herpes simplex virus 2 replication. Antiviral Res 215:105634. Research article – Q1
8. Bonzano S, Dallorto E, Molineris I, Michelon F, Crisci I, **Gambarotta G**, Neri F, Oliviero S, Beckervordersandforth R, Lie DC, Peretto P, Bovetti S, Studer M, De Marchis S (2023) NR2F1 shapes mitochondria in the mouse brain, providing new insights into Bosch-Boonstra-Schaaf optic atrophy syndrome. Dis Model Mech 16:dmm049854. Research article – Q2
9. Piccato A, **Crosio A**, Antonini A, Battiston B, Titolo P, Tos P, Ciclamini D (2023) Single-stage versus two-stage bone flap reconstruction in chronic osteomyelitis: Multicenter outcomes comparison. Microsurgery. Research article – Q2

5. Future directions and objectives for next years

a. Summary (up to 2000 characters):

The general goal of the research group will be to study innovative therapies to improve the patients' outcome after somatic and autonomic peripheral nerve lesions.
Both innovative and standardized in vitro and experimental in vivo models will be used to reach the goal.
In particular, glial and neuronal in vitro models will be used for the evaluation of innovative biomaterials that could be used as nerve repair prosthesis. Studies on GLUCIDEX®-derived membrane, dECM hydrogel and chitosan membranes functionalized will be continued for increasing our knowledge for a possible in vivo use.
Moreover, a "nerve-on-a-chip" system will be developed, as a new in vitro model for the study of nerve, using human induced pluripotent stem cells (hiPSC) cultured on a 3D scaffold developed by bioengineers at the Politecnico of Torino.
The nerve regeneration within different artificial or biological conduits used to repair a nerve gap will be further studied. In particular, decellularized porcine nerve grafts, 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 ability of support nerve regeneration, 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 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.
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):

<u>Improvement of the knowledge about the role of vascularization during nerve repair</u>
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 damage, 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".
<u>Development of human peripheral nerve models in vitro</u>

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 animals 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 after radical prostatectomy is still an important problem that affects patient's quality of life. The application of new techniques such as direct nerve transfer or membrane application would result in minor inconvenience for patients and allow extending the treatment also in the clinical oncological field.

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.

Studies on artificial or biological nerve devices

Biological scaffolds have attracted significant interest in the field of tissue engineering due to their great biocompatibility, bioactivity, the ability to moderate mechanical performances during nerve repair, and from a translational point of view.

Among various types of scaffolds, decellularized porcine nerve graft, decellularized extracellular matrix (dECM) hydrogel derived from cadaveric tissues and biomaterials, such as GLUCIDEX®-derived membrane and chitosan, have obtained great interest for different reasons.

Neuregulin α and β

After nerve injury and repair different Neuregulin1 (NRG1) isoforms are expressed and involved in Schwann cell dedifferentiation and differentiation. Both NRG1 α and β are expressed, but their roles have not yet been elucidated. To investigate their function in vivo, in collaboration with Zaragoza University, gold nanoparticles will be functionalized with polyethylene glycol and short interfering RNA (siRNA) to promote internalization and specific silencing of NRG1 α or β.

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

The general aim of the group is to study innovative strategies 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 life, including psychosocial and relational problems. 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 biological processes of nerve regeneration (role of endothelial cells, fibroblasts, schwann cells) and innovative strategies of tissue engineering on the peripheral nerve. These include the evaluation of innovative biomaterials that could be used as nerve repair prosthesis (Glucidex®-

derived membranes, dECM hydrogel, chitosan membranes functionalized) and biological conduits (decellularized pig nerve allografts, muscle-in-vein).

Developing a nanostructured chitosan medical device for its application in the urological clinical field. This objective will be pursued to further test nanostructured membranes to repair prostatic nerves in rats. Particularly, in the next future, we are planning to study the functionalization of the chitosan membrane with phosphodiesterase inhibitors to improve the performance of the device. The controlled release of phosphodiesterase inhibitors will be used to chemically promote nerve regeneration and functional recovery. In vitro experiments will be carried out to identify the device with the best performance for the following in vivo implantation on rats.

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 were: 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.

Aim 1 was obtained during the first year. Differentiation towards Schwann cells and sensitive neurons and points 2 and 3 will be addressed in 2024.

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

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 be translated to 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): <u>please fill-out this section only in the case of innovative technologies</u>

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2023

Laboratory name: **Ageing and Alzheimer's disease**

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2. CURRENT GRANTS

Starting-end date	Project Title and ID	Beneficiary	Funding Program/Agency	Role of the unit	Overall Amount Funded	Managed by FCO/UNITO
18/10/23 17/10/25	Extracellular vesicles derived from preconditioned Mesenchymal stem cell: a new challenge for AD therapy? Prot. 202229X8HW	Tamagno Elena	PRIN 2022	PI	67.911	UNITO
22/12/2023 30/06/2026	106831 - (2023.1759) - 'Studio di marcatori plasmatici precoci della malattia di Alzheimer: ruolo del miRNA218	Michela Guglielmotto	CRT Bando Richieste Ordinarie 2023	PI	30.000	UNITO

3. SCIENTIFIC ACTIVITIES IN 2023

Elena Tamagno, Role (PI)

Supervised PhD students:	Valeria Vasciaveo; Giulia Morello
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	Thomas Fenlz, Associate Professor, Clinic for Anesthesiology and Intensive Care TUM School of Medicine and Health Technical University of Munich; George Tetz, MD, PhD, Department of Systems Biology Human Microbiology Institute, NY.
• 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.):	

Michela Guglielmotto, Personnel

Supervised PhD students:	
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	Thomas Fenlz, Associate Professor, Clinic for Anesthesiology and Intensive Car, TUM School of Medicine and Health Technical University of Munich; George Tetz, MD, PhD, Department of Systems Biology Human Microbiology Institute, NY.
• Invited talks:	Seminar CNR 11/30/2023 Monza, PhD seminar: Doctoral school of Munich 11/16/2023
• Science communication:	
• Editorial duties:	
• others	
Organizational activities and responsibilities at NICO:	2nd Meeting GREEN NICO COMMITTEE, Green day 2023, responsible for the Bacteria Lab, Cell Culture Room Molecular Biology Lab and Western Blot Lab.
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

4. Research activity in 2023

a. Summary (500 characters)

Our group is involved in studying the cellular and molecular mechanisms associated with aging that cause Alzheimer's disease, 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. Several 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 of 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, 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 perivascular 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.
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.

Nutrition is also believed to be a modifiable environmental factor that could strongly impact on senile dementia and Alzheimer's disease (AD) pathology. Recent literature data demonstrated a protective role of individual food components, micro and macronutrients, in the prevention and progression of 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.

d. Results (4000 characters)

We obtained some interesting results regarding sleep fragmentation:

- 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 fragmentation compromises the object recognition memory in wild-type mice, indeed F-wt (F=Fragmentated) animals spent less time in the old object rather than in the new one compared with controls (NF= Not Fragmentated), but their total interaction with both objects is reduced respect to controls. Moreover, the two indexes of object recognition and discrimination were reduced in F-wt animals compared with controls. This decrease in object interaction may be due to increased stress after SF (Sleep Fragmentation).
  - Memory consolidation is lost following SF in the wt-type strain. To evaluate memory consolidation, we performed a series of tests, during which we observed that SF had a negative effect on memory consolidation. This was determined by analyzing the animals' behavior on the day following training. We assessed working memory (procedural memory) and problem-solving skills through the puzzle box (PB) test.
  - Sleep fragmentation can affect the glymphatic system by decreasing AQP4 levels in wild-type mice. In fact, we observed a significant decrease in AQP4-positive signal levels after SF in the amygdala and dentate gyrus, the two regions involved in learning, memory and sleep regulation. By analyzing GFAP-positive signal density, it was possible to show that the decrease in AQP4 was not caused by astrocytic cell death, as no significant changes were observed after sleep fragmentation.

- However, a correlation between the SF-mediated decrease in AQP4 and the deterioration of neuronal activity was demonstrated by measuring c-Fos-positive nuclei, which were found to be strongly decreased in brain areas where the decrease in AQP4 was observed.

We also obtained some interesting results regarding fast mimicking diet. The results are still very preliminary but seem to confirm a significant protection of the diet both on behavior and on the presence of neurofibrilary tangles.

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

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

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):

Alzheimer's disease (AD), the most common form of age-related dementia, is characterized by a progressive degeneration of the central nervous system (CNS) that leads to a gradual decline of cognitive functions and memory loss. Recently, preclinical, epidemiological, and genetic studies have demonstrated in neurodegenerative diseases, including AD, an earlier involvement of immune system. Since there is still no cure for AD, these studies motivated the design of innovative therapeutic strategies aiming at slowing down degenerative processes by targeting not only neuronal but also glial cells, both microglia and astrocytes, in virtue of their main recognized role in orchestrating neuroinflammatory process.

Mesenchymal stem cells (MSCs) are adult multipotent stem cells that over the last decades have been demonstrated to convey improvement in various models of neurodegenerative pathologies, thanks to their paracrine ability that is largely dependent on the secretion of extracellular vesicles

(EVs). The therapeutic potential of MSC-EVs, either as immunomodulators or as neuroprotective entities, has been recently put on focus also in the AD field. Moreover, the evidence that the intrinsic immunoregulatory abilities of MSCs is strongly influenced and strengthened by the environment, has led the scientists to design and optimize culture conditions (preconditioning) in order to enhance the anti-inflammatory properties of these cells and of their derived EVs. Thus, EVs produced by preconditioned human MSCs represent a promising therapeutic tool to limit or hamper neurodegeneration and inflammation in AD animal models. However, the neuroprotective and immunomodulatory mechanisms triggered by preconditioned MSC-EVs are still unclear and rather speculative.

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

Familial forms of AD (FAD) are caused by mutations or by overexpression of the APP gene, or by mutations or deletions in presenilin 1 and 2 (PSEN1/2) genes: the catalytic components of the proteolytic enzyme γ-secretase (GS). The "amyloid hypothesis" states that the aberrant processing of APP by GS induces the formation of specific neurotoxic soluble Amyloid-β (Aβ) peptides which, in turn, cause neuronal damage, brain inflammation, aggregation of the Microtubule Associate Protein Tau (MAPT) in intracellular neurofibrillary tangles (NFT) with axonal impairment. However, the "amyloid theory", has evidenced significant limitations since all attempts to develop Aβ-targeting drugs to treat AD have not succeeded. Recently, new preclinical, epidemiological and genetic studies have demonstrated a much earlier involvement of immune system-related actions, leading to a reassessment of the role of the principal innate immune entities of the brain, microglia cells.

Since at present, AD has non-reliable and available therapies, it becomes imperative to find out new treatments. In this context, mesenchymal stem cells (MSC) and their derived EVs (MSC-EVs) have been recently considered as a new powerful tool to contrast inflammation and neuronal degeneration in different pathological conditions, including AD. Recent data propose that most of the peculiar protective features of MSCs may be rather ascribed to the secretion of soluble factors, cytokines and, mainly, of EVs carrying immunomodulatory information. Many studies, including some of ours in AD, have already shown therapeutic effects exerted by MSC-EVs in animal models of different pathologies and few clinical trials have been published so far in which MSC-EVs were administered to patients.

Growing lines of evidence indicate that the use of MSCs, in the field of preclinical and clinical research, allows far greater advantages following their preconditioning with specific stimuli, aimed at enhancing the therapeutic actions of MSCs, as well as their derived EVs.

Many preconditioning strategies including modification of physical environment chemical/pharmaceutical reagents, biological factor and gene manipulations, have been applied to MSCs. Thus, preconditioned MSCs have shown improved therapeutic efficacy in different disease models, such as myocardial infarction, brain injury, colitis, graft-versus disease, and AD.

However, so far, a systematic analysis of the content of EVs derived from MSCs or from preconditionated MSCs has not been reported. In this scenario, the aims of this project fit well with the need to deepen EV mechanisms of action in order to understand their role in AD and to optimize their preclinical and clinical use. In particular, we will compare the EVs obtained with two different protocols of MSC preconditioning: Oxygen and Glucose Deprivation and proinflammatory cytokines (IFNγ and TNFα).

Concerning AD therapy, some recent research focused on the study of the role of the intestinal microbiome as a possible target. As a matter of fact, it has been described that an imbalance of the gut microbiome would facilitate the infiltration of peripheral immune cells into the brain, resulting in greater activation of microglia thus contributing to cognitive impairment and Aβ load in APP/PS1 mice . Interestingly, GV-971, a mixture of acidic linear oligosaccharides derived from brown algae, has completed a Phase 3 clinical trial

for AD in China and successfully met its primary endpoint in improving cognition impairment, becoming a new possible promise to improve cognition in Alzheimer's patients. After oral administration, most of the ingested GV-971 is retained in the gut. It is proposed that it can reconstitute the gut microbiota, reduce bacterial metabolite-driven peripheral infiltration of immune cells into the brain, and inhibit neuroinflammation in the brain as observed in animal models. Although GV-971 potential mechanism represents an AD-specific process is not clear, it is interesting to hypothesize that the combination of the modulation of the gut-brain axis via treatment with GV-971 and the modulation of neuroinflammation and neuroprotection in the brain via EVs should be a novel approach to slowdown the progression 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 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 investigating 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 present project aims to characterize the protective action of preconditioned MSC-EVs relatively to amyloid precursor protein (APP) processing and amyloid β (Aβ) formation, microtubule associated protein tau (MAPT) phosphorylation and aggregation, inflammation and activation of glial cells, the crucial hallmarks of AD.

We will focus on 4 main aspects:

1- proteomic and miRNA characterization of two different preconditioned MSC-EV populations;

2- challenging in vitro primary glial (microglia and astrocytes) and hippocampal neuronal cultures, obtained from transgenic mice (3xTg and hTau), with EVs obtained and characterized

3- treating in vivo 3xTg and hTau mice using EVs obtained and characterized

4- enhancing the protective effects of EVs by the in vivo co-administration of a new drug, GV-971, which affects the gut microbiome health.

Collectively, the results provide a realistic and promising opportunity to achieve new milestones in AD research and therapy.

Despite the success achieved in using MSCs in preclinical AD studies, their use in clinical applications requires further knowledge. In particular, in recent years, researchers have tried to find new strategies to overcome the obstacles encountered in clinical practice, standardizing the protocols regarding the number of cells, routes of administration and culture conditions.

One possibility could be the use of MSC-derived EVs. The identification of the specific protein/miRNA content in all types of MSC-derived EVs will lead to important pieces of information in the diagnostic and therapeutic fields. Indeed, as for the therapeutic purpose, the identification of the “beneficial” miRNAs or proteins and the correlated involved pathways will allow the development of a new therapeutic strategies able to modulate the identified pathways and the design of new engineered EVs. In the diagnostic field, the identification of the up-or down-regulation of specific protein/miRNA in different stages of the pathology may be also exploited as biomarkers for the early diagnosis of the disorders. Over the past two decades, there has been significant growth in the study of cerebrospinal fluid (CSF) biomarkers for AD, which diagnose the disease in the early stages. The aim of this field is to characterize new molecules which can be used as early predictive markers and can represent possible therapeutic targets capable of slowing the course of the disease. It is known that AD begins about 20 years before symptoms and therefore

we must exploit this long-time window to find early disease biomarkers that allow us to attack the disease at a very early stage.

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

even today the condition of Alzheimer's patients and their families appears to be for many ways emblematic of the difficulties of health and social care systems in providing adequate responses and solutions.

Dementia is on the rise in the general population and according to the World Health Organization and Alzheimer Disease International Report has been defined a world public health priority: "In 2010, 35.6 million people were affected by dementia with an estimated double increase in 2030, triple in 2050, with 7.7 million new cases per year (1 every 4 seconds) and with an average survival, after diagnosis, of 4-8 years.

The major risk factor associated with the onset of dementia is age and, in an aging society, the impact of the phenomenon is alarming in scale. These diseases are expected to quickly become one of the most significant problems in terms of public health.

AD is also a gender disease. Thus, female sex represents an important risk factor for the onset of AD, the most frequent form of all dementias (approximately 60%). The prevalence of dementia in industrialized countries is about 8% in the over 65s and rises to over 20% after the age of eighty.

According to some projections, cases of dementia could triple in the next 30 years in Western countries.

In Italy, according to demographic projections, in 2051 there will be 280 elderly people for every 100 young people, with an increase in all age-related chronic diseases, and among these dementias.

Currently the total number of patients with dementia is estimated at over 1 million (of which about 600,000 with AD) and about 3 million people are directly or indirectly involved in their care, with consequences also on an economic and organizational level.

AD certainly represents a social disease, in fact Alzheimer's could be considered a "family" disease, in the sense that the presence of a disease so progressively and deeply disabling, with many care needs it becomes an element disruptive within a family unit. Family can "get sick", because crushed by the weight of a commitment even psychologically heavy, involved in all aspects of care and assistance and able to count on the support of the services often extremely limited.

Economic impact: The cost estimate is $ 604 billion annually with steadily increasing and continuing challenge to health systems. All countries must include dementia in their public health programs. At the international, national, regional, and local levels, multi-level programs and coordination are needed and between all interested parties.

With the 2021 Budget Law, Italy established the "Alzheimer's and Dementia Fund", promising to allocate 15 million over three years. This allocation is certainly not sufficient with respect to the number of patients involved and the management cost of each individual patient. Thus, estimates on the costs of public and private Alzheimer's spending are around 70,000 euros per year, of which 73% are borne by Italian families.

Although enormous efforts, including economic ones, have been directed to attack this disease, AD remains inexorable and incurable, and the high rate of AD drug development failure may be due at least in part to our yet incomplete understanding of the complexes pathological mechanisms of this disease, hence the importance of basic studies.

Despite this, a cure for the disease must be found.

For decades, the "one drug for one target" strategy has been dominant, but it has not been able to defeat this multifactorial disease. It is therefore hypothesized that the multifunctional strategy, which could modify different pathological pathways at the same time, could be useful for treating this multifaceted pathology.
Collectively, the results that can be achieved in this project will provide a realistic and promising opportunity to achieve new milestones in AD research and therapy.
With respect to pharmacological therapies, although numerous research projects are currently underway to identify effective therapies in the treatment of dementia, the available interventions are not yet decisive. The therapeutic strategies available for dementia are pharmacological, psychosocial and integrated management for continuity of care. Especially for chronic degenerative pathologies such as dementias, therefore, it appears mandatory to define a set of care pathways according to an integrated management philosophy of the disease.

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 2023

Laboratory name: Neurophysiology of neurodegenerative diseases

1. LABORATORY DESCRIPTION – PERSONNEL:

Principal Investigator
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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	Funding Program/Agency	Role of the unit	Overall Amount Funded
2023-2025	Matrix Metalloproteinase-9 and PeriNeuronal Nets: new therapeutic targets for Fragile X Syndrome	Filippo Tempia	PRIN	component of Research Unit with Prof. Carola Eva	€ 51.445
2023-2025	Progetto:"Ruolo di GSK3 nei disturbi dell'umore	Filippo Tempia	Ricerca Locale UniTo	PI	€ 2.352
01/10/23-31/10/25	Atassia Telangiectasia: identificazione di meccanismi di neurodegenerazione affrontabili con trattamenti farmacologici	Eriola Hoxha	CRT	PI	€ 30.000
01/05/23-31/05/26	Deficit dei circuiti cerebellari in topi modello di Atassia-Teleangiectasia	Eriola Hoxha	Ricerca Locale UniTo	PI	€ 3.062

3. SCIENTIFIC ACTIVITIES IN 2023

Filippo Tempia (PI)

Supervised PhD students:	1: Anita Maria Rominto
• 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
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

Supervised PhD students:	1: Anita Maria Rominto
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 30th, 2023, in the frame of the meeting: "A-T Family Weekend 2023", September 29th to October 1st, 2023, Torre di Paestum (SA), Italy. Invited oral 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.
Organizational activities and responsibilities at NICO:	Responsible for the water ultrapurification systems at NICO
Technology transfer achievements (patents, etc.):	1 patent application (with Filippo Tempia)

4. Research activity in 2023

a. Summary (500 characters)

Following chronic social defeat stress, recognized as a murine model of depression, we found a dysregulation of GSK3 in the prefrontal cortex. Following tail suspension, a model of acute stress, we found increased neuronal activation, which was similar in wild type mice and in a model of resilience to depression. In a novel murine model of ataxia-teleangiectasia we found an enhanced cell death of Purkinje cells after an acute episode of hypoxia/hypoglycemia.

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. On the other hand, we recently proposed that deletion the cytoplasmic protein Fgf14 confers resilience to depression.
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.

c. Objectives (1000 characters)

Aim 1: Role of Gsk3 and Fgf14 in mood disorders.
Subaim 1.1: to find alterations of Gsk3 regulation in mice after induction of chronic social defeat stress.
Subaim 1.2: to investigate the neuronal activation in response to acute stress in mice with deletion of Fgf14
Aim 2. The goal was to detect neuronal cell death of cerebellar Purkinje cells in the animal model of AT.

d. Results (4000 characters)

Aim 1. Role of Gsk3 and Fgf14 in mood disorders. The aim of the project was 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 used the chronic social defeat stress (CSDS) paradigm. We showed that Gsk3β becomes hyperactive in mice susceptible to CSDS. After induction of a depressed-like behavior by CSDS, the active form of Gsk3 was significantly increased in the prefrontal cortex. We collected some recordings of neuronal activity in the PFC of wild type and $Gsk3^{-/-}$, and more data will be gathered in the next year of the project. In $Fgf14^{-/-}$ mice we found a similar neuronal activation in the PFC as in wild type controls.

Aim 2. The goal was to detect neuronal cell death of cerebellar Purkinje cells in the animal model of AT. Purkinje cell, in response to hypoxic/hypoglycemic stress, showed a massive dark cell degeneration in the animal model of AT, significantly higher than in control animals.

e. Advancement in the field (1000 characters)

Our results indicate that Gsk3 and Fgf14 are two important molecules involved in the response to acute and chronic stress, in animal models of depression. This opens new pharmacological perspectives for the treatment of depression.
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.

f. Publications

1. Ferrero E, Di Gregorio E, Ferrero M, Ortolan E, Moon YA, Di Campli A, Pavinato L, Mancini C, Tripathy D, Manes M, Hoxha E, Costanzi C, Pozzi E, Rossi Sebastiano M, Mitro N, **Tempia F**, Caruso D, Borroni B, Basso M, Sallese M, Brusco A (2023) Spinocerebellar ataxia 38: structure–function analysis shows ELOVL5 G230V is proteotoxic, conformationally altered and a mutational hotspot. Human Genetics. Published online 18 May 2023. https://doi.org/10.1007/s00439-023-02572-y

2. Rosso G, Maina G, Teobaldi E, Balbo I, Di Salvo G, Montarolo F, Rizzo Pesci N, Tempia F, Hoxha E. (2023). Differential diagnosis of unipolar versus bipolar depression by GSK3 levels in peripheral blood: a pilot experimental study. *Int J Bipolar Disord* **11**, 33 (2023). https://doi.org/10.1186/s40345-023-00314-7.

3. Lippiello P, Montarolo F, Tanaka-Yamamoto K, Hoxha E. (2023). The cerebellar involvement in non cerebellar pathologies. *Front. Cellular Neurosci*. doi: 10.3389/fncel.2023.1232155

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):

The neuronal mechanisms involved in mood control by Gsk3 and Fgf14 in murine models of depression will be investigated in the prefrontal cortex. Alterations in action potential firing will be studied in slices of medial prefrontal cortex of the murine models. In an animal model of AT, electrophysiological alterations of Purkinje cells will be studied by patch-clamp and calcium imaging. In an animal model of Fragile X syndrome, we aim at identifying new molecular targets and the most effective stage for a proper therapeutic intervention.

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.

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. Fragile X syndrome (FXS, OMIM #300624) is the most common monogenic cause of inherited intellectual disability and autism with an incidence of approximately 1:4000 males and 1:8000 females. FXS results from the CGG expansion at the 5'UTR of the FMR1 gene of >200 units and consequent gene silencing. FMRP protein, which is absent in FXS patients, is involved in multiple aspects of mRNA metabolism, particularly in the brain. No cure exists for FXS, and even approaches based on very promising targets (i.e. mGluR5 and GABA receptor inhibitors) failed to show clinical benefit, showing the difficulties to translate the experimental models into the clinic. Fmr1KO mice represent a useful model to study the effects of the lack of FMRP. Using Fmr1KO mice, a disorganization of perineuronal nets (PNNs) coupled with FXS-associated sensory hypersensitivity was demonstrated. PNNs are specialized forms of extracellular matrix (ECM), which arise in concomitance with the closure of developmental critical periods of plasticity and control synaptic connectivity and functions in the adult brain. An impairment of PNN formation around GABAergic parvalbumin-positive interneurons in the developing auditory cortex of Fmr1KO mice was observed and pharmacological or genetic restoration of PNNs significantly ameliorates FXS-associated hyperresponsivity to acoustic stimuli. However, little is known on FXS-dependent alterations of PNNs and their components affecting activity and plasticity of other brain areas that control cognition and emotions, i.e. hippocampus, prefrontal cortex and hypothalamus.

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 GSK3and FGF14, involved in mood disorders. Regarding neurologic diseases, we plan to focus on the neurophysiological basis of A-T and of Fragile X syndrome. 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 Fragile X syndrome.

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

Aim 1: Neuronal mechanisms involved in mood control by Gsk3 and Fgf14 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. In parallel, neuronal activity in the PFC will be studied in $Fgf14^{-/-}$ mice, which we showed to be resilient to depression.
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: The role of perineuronal nets (PNN) in the Fragile X syndrome. The goals of this project are to expand the current knowledge about the phenotype-genotype correlation in FXS and to identify new molecular targets and the most effective stage for a proper therapeutic intervention. The proposed strategy allows for preclinical assessment of new treatments of FXS at different developmental stages, opening experimental opportunities impossible to achieve with clinical studies.

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. No cure exists for FXS, and even approaches based on very promising targets failed to show clinical benefit, showing the difficulties to translate the experimental models into the clinic. Fmr1KO mice are a valid model to study new treatments. Novel pharmacological molecules targeted at PNN will be studied for effectiveness in FXS.

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

We plan to perform some of the measures of neuronal activity by in vivo two photon imaging.

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2023

Laboratory name: Brain development and disease

1. LABORATORY DESCRIPTION – PERSONNEL:

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

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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

Ferrero, Clelia, PhD student (since December 2023), Master's Degree in Neurobiology, 30/09/1997, +390116706632, clelia.ferrero@unito.it, Amyotrophic lateral sclerosis, stem cells, iPSCs, neurodegeneration

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Veloz-Castillo, Maria Fernanda, PhD student, Master degree in BioScience, 03/01/1995, +393519226321, mariafernanda.velozcastillo@unito.it, Brain energy metabolism, behavior, image analysis

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2. CURRENT GRANTS

Starting-end date	Project Title and ID	Beneficiary	Funding Program/Agency	Role of the unit	Overall Amount Funded	Managed by FCO/UNITO
2023	SMA	Vercelli A.	Girotondo Onlus	Coordinator	15,000 €	FCO
2021-24	Evaluation of new compounds to sustain muscular innervation and trophism	Vercelli A./Boido M.	Pharmafox – M. Hildigher	Coordinators	80,000 €	FCO
2023-2024	Eloquent areas in the CC of patients undergoing glioma surgery	Vercelli A./Garbossa D.	Reply	Coordinator	100,000	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
2021-2024	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
2023-2027	Department of Excellence	Vercelli A.	MUR	Coordinator DNS - UNITO	7.75 M€	UNITO
2023-2025	Understanding and targeting Chemotherapy-related neurotoxicity	Vercelli A.	PRIN 2022	P.I. of research unit	85,000 €	UNITO
2023-2026	Innova	Vercelli A	PNRR – Ministry of Health	Coordinator UNITO	279,850	UNITO
2019-2023	The role of SMN protein in translation: implications for Spinal Muscular Atrophy; ID GGP19115A	Boido M.	Fondazione Telethon	P.I. of research unit	83,600 €	UNITO
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 predittiva e di precisione nel neurocovid	Boido M.	Regione Piemonte (INFRA-P realizzazione, rafforzamento e ampliamento	P.I. of research unit	50,000 €	FCO

			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.	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	30,000 €	UNITO
2021-2024	Validazione preclinica di protocolli di inoculo di cellule staminali neurali umane (hNSCs) per lo sviluppo di terapie cellulari per pazienti affetti da sclerosi laterale amiotrofica	Boido M	Fondazione Revert	Coordinator	41,000 €	FCO
2023-2025	A 3D Bioprinted spinal cord Model to reach functional meaningful and clinically translatable regeneration	Boido M.	PRIN 2022	Coordinator	61,711 €	UNITO
2021-2024	NODES - TINCARE	Calì C.	PNRR	P.I. of the research unit	30,000 €	UNITO
2023	Grant for Internationalization	Calì C.	UNITO	P.I.	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 control gene regulation in importin alpha3 knock-out mice.	Marvaldi L.	Programma per Giovani Ricercatori “Rita Levi Montalcini”	Coordinator	251,581 €	UNITO

2022-2023	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
2022-2024	Braccio di ferro con la demenza: ferro e mitocondri come nuovi target contro la Malattia di Alzheimer	Stanga S.	Fondazione CRT	P.I.	20,000 €	FCO
2023	Study of human iPSC-derived neural stem cells to unravel molecular mechanisms related to early mitochondrial dysfunctions and iron dyshomeostasis in neurodegenerative diseases	Stanga S.	Internazionalizzazione 2022 - UNITO	P.I.	15,233 €	UNITO
2023	New strategy to prevent the progression of neurodegeneration occurring in Alzheimer's disease.	Stanga S.	Internazionalizzazione 2022 - UNITA	P.I.	12,500 €	UNITO
2023-2025	Targeting mACONITASE and KIF5a to rescue mitochondrial mobility and function for the treatment of motor neuron diseases	Stanga S.	PRIN 2022	Coordinator	102,625 €	UNITO
2023-2025	Evaluation of the effect of leriglitazone on brain iron deposits in a murine model of Alzheimer's disease	Stanga S.	Minoryx therapeutics	P.I.	60,000 €	FCO

3. SCIENTIFIC ACTIVITIES IN 2023

Alessandro Vercelli, PI

Supervised PhD students:	A. Caretto (co-tutorship with M. Boido), S. Dallere, G. Pavarino
Honors, prizes, awards:	
Outreach activities	

• International collaborations:	G. Aumayr (Austria), M. Summers (Australia), Makoto Sato (Osaka University, Japan), Ryuta Kawashima (Tohoku, Japan), Helena Vieira (Lisboa, Portugal)
• Invited talks:	Da MyAHA al verde/blu: terapie non farmacologiche per il sistema nervoso Università di Foggia (25/1/2023) L'insegnamento dell'anatomia pre e postlaurea, dal micro al macro e dalla realtà virtuale al cadaver lab. Accademia di Medicina Torino (7/2/2024) Commemorazione del 500° anniversario della nascita di Gabriele Falloppio a Modena. Opening ceremony of the Meeting of the Italian Society of Anatomy and Embryology. The appearance of von Economo's neurons in the mammalian brains: a clue in the evolution of the "social brain" and self-awareness. University of Trieste (26/9/2023) Evoluzione di una disciplina: come cambia l'insegnamento dell'Anatomia. Congresso SIPEM Torino (28/10/2023) Neuroanatomia: il contributo degli scienziati torinesi. OMCEO Torino (25/22/2023)
• Science communication:	Public Lecture: "The Brain in a Forest," regarding the exhibition "The Mountain Touch." National Mountain Museum "Duca degli Abruzzi"- Italian Alpine Club - Turin Section (together with Boido M. and Schellino R.). Turin (26/01/2023) Presentazione del NICO Praxi. Generations Brain@work Torino (6/2/2023) I meccanismi della visione. Fondazione Faraggiana Novara (28/2/2023) Le neuroscienze al NICO. Collegio Valsalice Torino (8/5/2023) I grandi temi delle neuroscienze in Italia e nel mondo – Evento di Chiusura delle Olimpiadi delle neuroscienze – Napoli (12/5/2023) L'intelligenza delle emozioni. come le emozioni ci aiutano a vivere. Liceo Classico Cavour (21/4/2023) Intelligenza artificiale e neuroscienze. Premio Morrione, Circolo dei Lettori (27/10/2023)
• Editorial duties:	Associate Editor Frontiers Ageing Neuroscience
• others	
Organizational activities and responsibilities at NICO:	Scientific Director
Speakers invited:	Fernando De Castro (Cajal Institute, Spain), Francesco Moneta (Bruker BioSpin)
Other organizational activities:	Deputy Rector for Biomedical Research, Director Neuroscience Institute of Turin, representative of UNITO in R4L (research for life), vice director for research DNS
Workshops, Schools or Conferences organized:	SINS 2023 (President of the Italian Society of Neuroscience)
Technology transfer achievements (patents, etc.):	President Spin-off committee, UNITO; UNITO representative in European Institute of Innovation & Technology Health

Marina Boido, Associate Professor

Supervised PhD students:	A. Caretto (co-tutorship with A. Vercelli), C. Ferrero, DM Rasà, F. Virla (co-tutorship with R. Mariotti, UNIVR)
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	Prof. Artero (Univ. Valencia), Prof. Soler (University of Lleida, Spain), Pharmafox Therapeutics AG (Switzerland), Dr. Martinat (I-STEM, Corbeil-Essonnes, France), P. Smeriglio (Institut de Myologie, Paris, France), A. Prochiantz (Collège de France, Paris, France), Prof. Cristovao (Univ. Beira Interior, Portugal)
• Invited talks:	Invited seminars: "Spinal muscular atrophy: from the pathogenetic mechanisms to the therapies". IDOR, Rio de Janeiro (05/10/2023) "Motor neuron diseases: from the pathogenetic mechanisms to the therapies". ICB-UFRJ (06/10/2023) Invitation upon selection at meetings: Boido M, Gesmundo I, Caretto A, Pedrolli F, Schellino R, Leone S, Cai R, Sha W, Ghigo E, Schally AV, Vercelli A, Granata R. "The therapeutic effects of MR-409, a GHRH agonist, in an experimental mouse model of spinal muscular atrophy". 76th National Congress of the Italian Society of Anatomy and Histology (S.I.A.I), Modena, Italy (11-13/09/2023) Boido M, Gesmundo I, Caretto A, Pedrolli F, Schellino R, Leone S, Cai R, Sha W, Ghigo E, Schally AV, Vercelli A, Granata R. "Investigating a new therapeutic role of the GHRH agonist MR-409 in an experimental model of spinal muscular atrophy". 20th National Congress of the Italian Society of Neuroscience (SINS). Turin, Italy (14-17/09/2023) Boido M, Gesmundo I, Caretto A, Pedrolli F, Schellino R, Leone S, Cai R, Sha W, Ghigo E, Schally AV, Vercelli A, Granata R. "Agonist of growth hormone-releasing hormone improves the disease features of spinal muscular atrophy mice". Neuroscience 2023, Washington, DC, USA (11-15/11/2023)
• Science communication:	Public Lecture: "The Brain in a Forest," regarding the exhibition "The Mountain Touch." National Mountain Museum "Duca degli Abruzzi"- Italian Alpine Club - Turin Section (together with Vercelli A. and Schellino R.). Turin (26/01/2023) Scientific Seminar (online) for the series "Sciencyclops_ an eye for science" of Merck Serono Group, Science Network Ivrea, Colleretto Giacosa (TO). Presentation to Merck Serono groups in Europe; Presentation title: "Spinal Muscular Atrophy: from the bench to the bedside ... and back again" (together with Schellino R.) (10/03/2023) Regional coordinator (Piedmont) of Olympics in Neuroscience; Regional stage, Turin (18/03/2023) Seminar "SMA, una malattia genetica dell'infanzia: come la ricerca sa trovare le soluzioni" at IIS Santorre di Santarosa (18/05/2023)

• Editorial duties:	Guest Associate Editor in Frontiers in Neuroanatomy. Research Topic: "New Insights in the Cell Mechanisms Involved in Neuromuscular Diseases: From the CNS to the Endplate" (together with Schellino R.)
• Others	General Secretary in the SIBS directive board; Coordinator of CU6 in the national PhD "Sustainable Development and Climate Change"; Representative of the Italian Society for Neuroscience to the ALBA Network
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:	Marta Valenza (University of Milan); Ana Clara Cristovao (University of Beira Interior, Covilhã, Portugal; together with S. Bovetti and S. Stanga)
Other organizational activities:	CEO of S&P BRAIN SRL
Workshops, Schools or Conferences organized:	Member of the Local Organizing Committee of the 20th National Congress of the Italian Society of Neuroscience (SINS), Turin, Italy (14-17/09/2023) Chair and organizer of the symposium entitled: "Beyond motor neurons: skeletal muscle contribution in motor neuron diseases", 20th National Congress of the Italian Society of Neuroscience (SINS), Turin, Italy (15/09/23)
Technology transfer achievements (patents, etc.):	na

Corrado Calì, Associate Professor

Supervised PhD students:	M. F. Veloz Castillo, V. Chiappini
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	Pierre Magistretti (KAUST, Saudi Arabia), Marco Agus (Hamad Bin Khalifa University, Qatar), Markus Hadwiger (KAUST, Saudi Arabia)
• Invited talks:	Annual meeting of Portuguese Glial Network; Seminar Series, HBKU, Doha, Qatar; 20th year meeting of the Brain and Mind Institute, EPFL
• Science communication:	Life of a Learning Neuron, Brain Awareness Week 2023 Ricercatori alla Spina
• Editorial duties:	Associate editor of Advanced Technology in Neuroscience
• others	na
Organizational activities and responsibilities at NICO:	na
Speakers invited:	Christel Genoud (University of Lausanne); John Mitrofanis (Fonds Clinatec, Grenoble)
Other organizational activities:	na
Workshops, Schools or Conferences organized:	Member of the Local Organizing Committee of the 20th National Congress of the Italian Society of Neuroscience (SINS), Turin,

	Italy (14-17/09/2023); 20th National Congress of the Italian Society of Neuroscience (SINS), Chair and organizer of 3DEM Symposium; Organizer of geometric computational models of astrocytes Symposium at FENS Regional Meeting, Algarve, Portugal
Technology transfer achievements (patents, etc.):	na

Letizia Marvaldi, Assistant Professor RTD-B

Supervised PhD students:	A. Parmar
Honors, prizes, awards:	Rita Levi Montalcini Fellowship (MIUR)
Outreach activities	
• International collaborations:	Dr. Franziska Rother (MDC Berlin)
• Invited talks:	Gordon Conference Neurotrophins Factors Function in Development, Plasticity and Disease. Newport, RI, USA (30/05/2023) Pathophysiology and genetics of neuron and muscle (PGNM). Institute NeuroMyogene, Lyon, France (12/12/2023)
• Science communication:	U*NIGHT 2023 (30/09/2023) Unistem Day (10/03/2023)
• Editorial duties:	na
• others	Poster at meeting: Poster Presentation at the EMBO workshop mechanism of neuronal remodelling". Title of the poster: "Aberrant axon morphology in an importin mutant mouse". Kibbuz Nahsholim, Israel (March 11-14, 2023)
Organizational activities and responsibilities at NICO:	Coordination for BioRender license (2023-2024)
Speakers invited:	Dr. Indrek Koppel Taltech (Estonia); Prof. Mike Fainzilber Weizmann Institute (Israel)
Other organizational activities:	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Roberta Schellino, Assistant Professor RTD-B

Supervised PhD students:	na
Honors, prizes, awards:	National Scientific qualification as associate, Academic Recruitment Field 05/H - Human anatomy, 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
Outreach activities	
• International collaborations:	J. Willem Vrijbloed (Pharmafox Therapeutics AG); R. Fariello (Pharmafox Therapeutics AG)
• Invited talks:	Invitation upon selection at meetings:

	Schellino R, Menduti G, Boido M, Vercelli A. “Alteration of projection neurons in a murine model of spinal muscular atrophy suggests a remodelling in cortical cytoarchitecture due to SMN lack”. 95th Congress of the Italian Society of Experimental Biology (S.I.B.S.), Trieste, Italy (12-15/04/2023) Schellino R, Boido M, Vrijbloead JW, Fariello R, Vercelli A. “ActR-Fc-nLG3: a novel biological to increase neuromuscular junction stability and endurance in old sarcopenic mice, by synergistically acting on myostatin and agrin pathways”. 76th National Congress of the Italian Society of Anatomy and Histology (S.I.A.I), Modena, Italy (11-13/09/2023)
• Science communication:	Public Lecture: "The Brain in a Forest," regarding the exhibition "The Mountain Touch." National Mountain Museum "Duca degli Abruzzi"- Italian Alpine Club - Turin Section (together with Vercelli A. and Boido M.). Turin (26/01/2023) Scientific Seminar (online) for the series "Sciencyclops_ an eye for science” of Merck Serono Group, Science Network Ivrea, Colleretto Giacosa (TO). Presentation to Merck Serono groups in Europe; Presentation title: "Spinal Muscular Atrophy: from the bench to the bedside ... and back again" (together with Boido M.) (10/03/2023) Member of the local organizing committee at “Olimpiadi delle Neuroscienze”, Torino (19/03/2023) Online interview for the MeetScience channel. Interview title: "Stem cell transplants for the treatment of neurodegenerative diseases." Interview streamed and viewable on YouTube on MeetScience channel (05/04/2023)
• Editorial duties:	Guest Associate Editor in Frontiers in Neuroanatomy. Research Topic: “New Insights in the Cell Mechanisms Involved in Neuromuscular Diseases: From the CNS to the Endplate” (together with Boido M.) Review board for the journals: Frontiers in Neuroscience (ISSN 1662-453X), Frontiers group; 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	Participation as selected member at the NSAS Advanced Course “Organoid modeling and neural reprogramming”, Venice, Italy (13-20/05/2023) Poster at meeting: Schellino R, Boido M, Vrijbloed JW, Fariello R, Vercelli A. “ActR-Fc-nLG3: a novel biological sustaining neuromuscular junction innervation in sarcopenia”. 20th National Congress of the Italian Society of Neuroscience (SINS). Turin, Italy (14-17/09/2023)
Organizational activities and responsibilities at NICO:	Responsible for E800 Nikon Eclipse fluorescence microscope

Speakers invited:	Michela Rigoni and Samuele Negro (University of Padua, Italy); Chrystian Junqueira Alves (Icahn School of Medicine at Mount Sinai, New York)
Other organizational activities:	Chair at the 20th National Congress of the Italian Society of Neuroscience (SINS), for the under40 symposium "Neuroinflammation in the basal ganglia networks: implications for neurological diseases". Turin, Italy (16/09/23)
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:	IBRO Collaborative Research Grant program for the building of a collaboration with the group lead by Prof. Clive Svendsen in Cedars Sinai, Los Angeles (CA, USA) for 2023-2024 Premialità RTD-B 2023 from Università degli studi di Torino
Outreach activities	
• International collaborations:	Prof. Pascal Kienlen-Campard (UCLouvain, Bruxelles Belgium); Dr. Emilie Auduard and Prof. Francoise Piguet (Inserm, Paris, France); Prof. Alain Prochiantz and Dr. Ariel Di Nardo (College de France, Paris, France); Prof. Clive Svendsen, Dr. Deepti Lall and Dr. Frank Diaz (Cedars-Sinai, Los Angeles, USA); Prof. Ana Clara Cristovao (University of Beira Interior, Covilhã, Portugal) International Visiting Scientist by virtue of the Grant for Internationalization 2023 (GFI 2023), in the laboratory of Development and Neuropharmacology / LabCom team directed by Ariel Di Nardo and Alain Prochiantz at Collége de France, Paris (March-April 2023) International Visiting Scientist by virtue of the IBRO Collaborative Research Grant, for the building of a collaboration with the group lead by Prof. Clive Svendsen in the Regenerative Medicine Center (RMI), Cedars Sinai, Los Angeles, USA (August-November 2023)
• Invited talks:	Invited seminars: Seminar at College de France "Brain iron dyshomeostasis: a red thread across aging and neurodegenerative diseases", Paris, France (30/03/23) Webinar for the Amici della Morfologia "Methods and tools for the quantitative analysis of mitochondrial morphology and network in tissue and cells" (06/05/23) Seminar at RMI, Cedars-Sinai, USA "Brain iron dyshomeostasis and mitochondrial alterations as red threads across aging and neurodegenerative diseases" (17/11/23) Invitation upon selection at meeting:

	"Brain iron accumulation: a shared hallmark of aging and Alzheimer's disease?". 20th National Congress of the Italian Society of Neuroscience (SINS). Turin, Italy (14-17/09/2023)
• Science communication:	Meeting and guided tour of NICO with EMSA students (European Medical Students´ Association) (09/03/23) Invited seminar EMSA students (European Medical Students´ Association) San Luigi, Orbassano "In pursuit of healthy brain aging: unveiling the biology of novel age-related mechanisms leading to dementia" (26/06/23) Intervista Trend Sanità giornata mondiale Alzheimer "La ricerca e le nuove sfide per la cura dell'Alzheimer" (21/09/23)
• Editorial duties:	External expert for the European Commission, Evaluations for HORIZON MSCA 2022-DN-01 Review editor for Cellular and Molecular Life Sciences (CMLS), I completed n=3 editorial contributions in 2023
• others	na
Organizational activities and responsibilities at NICO:	Responsible for the Cell Culture room (floor 0) Responsible for the dissection room (floor -1) Member of the Green Policy Committee
Speakers invited:	Alain Prochiantz (College de France, Paris, France); Alessandro Ferrarini (STARLAB); Ana Clara Cristovao (University of Beira Interior, Covilhã, Portugal; together with M. Boido and S. Bovetti)
Other organizational activities:	Organization of the Lab meetings 2023 of the group: Brain development & disease Member of the Local Organizing Committee of the 20th National Congress of the Italian Society of Neuroscience (SINS), Turin, Italy (14-17/09/2023) Chair and organizer of the symposium entitled: "In pursuit of healthy brain aging: unveiling the biology of novel age-related mechanisms leading to dementia", 20th National Congress of the Italian Society of Neuroscience (SINS), Turin, Italy (16/09/23) Initiator of the NICO Green Policy Committee in 2023
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Mariarosa Mezzanotte, post-doc

Supervised PhD students:	na
Honors, prizes, awards:	Assegno di Ricerca, Department of Neuroscience "Rita Levi Montalcini", University of Torino. Project title: "Studio del metabolismo cerebrale del ferro e della funzionalità mitocondriale in modelli cellulari e murini di Malattia di Alzheimer" Winner of IBRO travel grant for participation to ESN-ISN Neurochemistry School (Targeting molecular mechanisms

	underlying neurodegeneration: focus on advanced therapies) in Faro, Portugal from 29/10/23 until 4/11/23
Outreach activities	
• International collaborations:	Prof. Ana Clara Cristovao (University of Beira Interior, Covilhã, Portugal) International Visiting Researcher, Health Sciences Research Center Faculty of Health Sciences University of Beira Interior (UBI), Covilhã, Portugal, under the supervisor of Prof. Ana Clara Cristovao: "New strategy to prevent the progression of neurodegeneration occurring in Alzheimer's Disease" in the context of the collaboration with UBI financially supported by the project UNITA: Aging population - Grant for Internationalization, PI Dr. Serena Stanga, from 08/05/2023 until 02/06/2023
• Invited talks:	Invitation upon selection at meeting: Mezzanotte M, Ammirata G, Boido M, Roetto A, Stanga S. "Brain iron deposition during aging and neurodegenerative diseases". 95th National Congress of the Italian Society for Experimental Biology, Trieste, Italy (12-15/04/23) Invited seminar: "Brain iron accumulation during aging and Neurodegenerative diseases". CICSUBI Seminar, Health Sciences Research Center, University of Beira Interior (UBI), Covilhã, Portugal (01/06/23)
• Science communication:	Participation in the scientific dissemination session: "Ricercatori alla Spina" Brain Edition - Centro Scienze; Turin, Italy (17/03/2023) Member of the local organizing committee at "Olimpiadi delle Neuroscienze", Torino (19/03/2023)
• Editorial duties:	na
• others	Posters at meetings: Mezzanotte M, Ammirata G, Boido M, Roetto A, Stanga S. "Age-dependent brain iron overload causes Hepc/Fpn1 pathway activation and the astrocytic-neuronal crosstalk" FENS regional meeting, Algarve, Portugal (3-5/05/23) Mezzanotte M, Ammirata G, Boido M, Roetto A, Stanga S. "Hepcidin/Ferroportin1 axis activation and astrocytic-neuronal crosstalk in C57BL/6 mice brain during aging". 20th National Congress of the Italian Society for Neuroscience (SINS), Torino, Italy (14-17/09/2023) Mezzanotte M, Ammirata G, Boido M, Roetto A, Stanga S. "Iron accumulation in the brain: focus on aging and neurodegenerative diseases". ESN-ISN Neurochemistry School Algarve, Faro, Portugal (02/11/23)
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:	na
Workshops, Schools or Conferences organized:	na

Technology transfer achievements (patents, etc.):	na

Giovanna Menduti, Post-doc

Supervised PhD students:	na
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	na
• Invited talks:	Invitation upon selection at meeting: Menduti G, Januel C, Ruatti C, Berenger-Currias N, Martinat C, Artero R, Konieczny P. Boido M. "Uncovering repositioned therapies efficacy for Spinal Muscular Atrophy treatment", 20th National Congress of the Italian Society for Neuroscience (SINS), Torino, Italy (14-17/09/2023)
• Science communication:	Member of the local organizing committee at "Olimpiadi delle Neuroscienze", Torino (19/03/2023)
• Editorial duties:	na
• others	Poster at meeting: Menduti G, Beltrando G, Vercelli A*, Boido M*. "GABA signalling and metabolism (dys)regulation in Spinal Muscular Atrophy: investigations in SMAΔ7 mice sensorimotor cortex", Neuroscience 2023, Washington, DC, USA (11-15/11/2023)
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

Livia Testa, Post-doc

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	na
• Invited talks:	Invitation upon selection at meeting: "The role of importin alpha 3 contribution to the pain transduction pathway", 20th National Congress of the Italian Society for Neuroscience (SINS), Torino, Italy (14-17/09/2023)
• Science communication:	na
• Editorial duties:	na
• Others	Poster at meeting: Gordon Conference Neurotrophins Factors Function in Development, Plasticity and Disease. "The role of importin alpha

	3 contribution to the pain transduction pathway". Newport, RI, USA (28/05/2023-02/06/2023)
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

Anna Caretto, PhD Student

Supervised PhD students:	na
Honors, prizes, awards:	2nd "Enrica Marzola Award" winner for the Basic Sciences curriculum of the PhD Course in Neuroscience (Unito)
Outreach activities	
• International collaborations:	Prof. Alain Prochiantz (Paris, France)
• Invited talks:	Invitation upon selection at meeting: Caretto A, Boido M, Gesmundo I, Pedrolli F, Schellino R, Leone S, Cai R, Sha W, Ghigo E, Schally AV, Vercelli A, Granata R. "Agonist of Growth hormone-releasing hormone improves the disease feature of Spinal Muscular Atrophy mice". 95th SIBS National Congress, Trieste (12-15/04/2023)
• Science communication:	Member of the local committee for "Pint of Science". Torino (22-24/05/2023) Member of the organizing committee for the stand "Accendi il cervello" in the Play Area for the U*NIGHT event, Torino (30/09/2023)
• Editorial duties:	na
• others	Posters at meetings: Caretto A, Di Cunto F, Boido M, Vercelli A. "Analysis of possible glycinergic system alterations in Spinal Muscular Atrophy". National meeting of PhD Students in Neuroscience. Torino, Italy (14/09/2023) Caretto A, Di Cunto F, Boido M, Vercelli A. "Analysis of possible glycinergic system alterations in Spinal Muscular Atrophy". 20th National Congress of the Italian Society for Neuroscience (SINS), Torino, Italy (14-17/09/2023)
Organizational activities and responsibilities at NICO:	Responsible for the electrophoresis room
Speakers invited:	na
Other organizational activities:	na
Workshops, Schools or Conferences organized:	Organization of the SINS "PhD Student National Meeting in Neuroscience", Torino, Italy (14-17/09/2023)
Technology transfer achievements (patents, etc.):	na

Sveva Dallere, PhD student

Supervised PhD students:	na
Honors, prizes, awards:	Second prize at PhDay, IUSS, with the poster "The effect of green exposure in the prevention and treatment of Alzheimer's disease: preliminary in vitro study". Pavia (13/06/2023)
Outreach activities	
• International collaborations:	Willem Vrijbloed (Pharmafox Therapeutics AG); R. Fariello (Pharmafox Therapeutics AG)
• Invited talks:	na
• Science communication:	Member of the local organizing committee at "Olimpiadi delle Neuroscienze", Torino (19/03/2023) Presenter of the scientific menu "Più attivi, meno smemorati" at Ricercatori alla Spina, Torino, Italy (18/05/2023) Poster presenter at U*NIGHT – Notte Europea delle Ricercatrici e dei Ricercatori 2023, Turin, with the poster "L'attività fisica all'aperto come prevenzione e terapia per la malattia di Alzheimer" (30/09/2023)
• Editorial duties:	na
• others	Posters at meetings: Dallere S, Boido M, Vercelli A. "The effect of green exposure in the prevention and treatment of Alzheimer's disease: preliminary in vitro study", European Neuroscience Conference by Doctoral Students (ENCODS), Faro, Portugal (01-02/05/2023) Dallere S, Boido M, Vercelli A. "The effect of green exposure in the prevention and treatment of Alzheimer's disease: preliminary in vitro study" PhDay, IUSS Pavia, Italy (13/06/2023) Dallere S, Signorino E, Pavarino G, Ferrero C, Boido M, Vercelli A. "Terpenes and Alzheimer's disease: a preliminary in vitro study", 20th National Congress of the Italian Society for Neuroscience (SINS), Torino, Italy (14-17/09/2023)
Organizational activities and responsibilities at NICO:	Member of the Green Policy Committee
Speakers invited:	na
Other organizational activities:	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Clelia Ferrero, PhD student (since December 2023)

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	na
• Invited talks:	na
• Science communication:	Member of the local organizing committee at "Olimpiadi delle Neuroscienze", Torino (19/03/2023)
• Editorial duties:	na

• others	Posters at meeting: "Intracerebroventricular hNSC graft in ALS mice: first pre-clinical results". 20th National Congress of the Italian Society for Neuroscience (SINS), Torino, Italy (14-17/09/2023)
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

Amishaben Parmar, PhD Student

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	Dr. Franziska Rother, MDC Berlin
• Invited talks:	Na
• Science communication:	na
• Editorial duties:	na
• Others	Posters at meetings: "The role of importin alpha 3 in sensory neurons upon in vitro injury", Gordon Conference Neurotrophins Factors Function in Development, Plasticity and Disease. Newport, RI, USA (28/05/2023-02/06/2023) "The role of importin alpha 3 in sensory neurons upon in vitro injury", 20th National Congress of the Italian Society of Neuroscience (SINS), Torino, Italy (14-17/09/2023)
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

Gianna Pavarino, PhD student

Supervised PhD students:	na
Honors, prizes, awards:	"Best ESN poster award, first place" for the best poster presentation "Health benefits of living in close proximity to greenery: preliminary results on depression". ENCODS 2023, Faro, Portugal (01-02/05/2023)
Outreach activities	

• International collaborations:	na
• Invited talks:	na
• Science communication:	Member of the local organizing committee at "Olimpiadi delle Neuroscienze", Torino (19/03/2023) Presenter of the scientific menu "Il cervello in un bosco" at the dissemination event "Ricercatori alla spina - Brain edition", Turin, Italy (18/05/2023)
• Editorial duties:	na
• others	Posters at meetings: Pavarino G, Brasso C, Schellino R, Carluccio A, Baire S, Signorino E, Boido M, Rocca P, Vercelli A. "The biological role of "green-therapy": a study on depressed patients". 20th National Congress of the Italian Society of Neuroscience (SINS), Torino, Italy (14-17/09/2023) Pavarino G, Brasso C, Schellino R, Carluccio A, Baire S, Signorino E, Boido M, Rocca P, Vercelli A. "The biological role of "green-therapy": a study on depressed patients". SINS National Meeting of PhD Students in Neuroscience 2023. Turin, Italy (14/09/2023) Pavarino G, Brasso C, Schellino R, Carluccio A, Baire S, Signorino E, Boido M, Rocca P, Vercelli A. "Health benefits of living in close proximity to greenery: preliminary results on depression". ENCODS 2023. Faro, Portugal (01-02/05/2023)
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

Vanessa Chiappini, PhD student (since November 2023)

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	na
• Invited talks:	na
• Science communication:	Member of the local organizing committee at "Olimpiadi delle Neuroscienze", Torino (19/03/2023)
• 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

Daniela Maria Rasà, PhD student

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	na
• Invited talks:	na
• Science communication:	Member of the local organizing committee at "Olimpiadi delle Neuroscienze", Torino (19/03/2023)
• Editorial duties:	na
• others	Posters at meetings: Rasà DM, Stoppa I, Boido M. "Possible correlation between stressors and amyotrophic lateral sclerosis onset and progression: in vitro preliminary results", 95th Congresso Nazionale della Società Italiana di Biologia Sperimentale, Trieste, Italy (12-15/04/2023) Rasà DM, Stoppa I, Boido M. "A study to clarify correlations between stressors and Amyotrophic Lateral Sclerosis", PhDay, Pavia, Italy (13/06/2023) Rasà DM, Stoppa I, Boido M. "Can stressors affect ALS onset and progression? Preliminary observations", PhD student National Meeting in Neuroscience, Torino, Italy (14/09/2023) Rasà DM, Stoppa I, Boido M. "Can stressors affect ALS onset and progression? Preliminary observations", 20th National Congress of the Italian Society for Neuroscience (SINS), Torino, Italy (14-17/09/2023) Rasà DM, Stoppa I, Boido M. "Unravelling the correlation between stressors and amyotrophic lateral sclerosis onset in an in vitro experimental model", Neuroscience 2023, Washington, DC, USA (11-15/11/2023)
Organizational activities and responsibilities at NICO:	Member of the Green Policy Committee
Speakers invited:	na
Other organizational activities:	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Ersilia Nicorvo, Fellowship recipient

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

• Invited talks:	na
• Science communication:	Member of the local organizing committee at "Olimpiadi delle Neuroscienze", Torino (19/03/2023)
• Editorial duties:	na
• others	Poster at meeting: Nicorvo E, Rasà D, Stanga S, Santonicola P, Montefusco S, Medina D, Vercelli A, Di Schiavi E, Boido M. "New SMN-independent drugs for Spinal Muscular Atrophy treatment". 20th National Congress of the Italian Society for Neuroscience (SINS), Torino, Italy (14-17/09/2023)
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

4. Research activity in 2023

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 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 and drug repositioning approaches in preclinical models. 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.

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 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. Recently, in the frame of our participation to the Italian PNRR projects, we are involved in the creation of a IPCs and organoid platform to model neural development and disease, and to test new drugs in an in vitro setting. We are also involved in the search for biomarkers of disease, both to predict and to study the

progression. 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. In particular, we are improving our technology to study the microscale with 3D EM, in strict collaboration with Zeiss. 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 spinal cord, and their circuitry, as substrate for CNS 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. Moreover, we study the neuronal death mechanisms and neuroinflammation during development and disease, and how to prevent/modulate them.
In collaboration with clinicians, we are also interested in a) the effect of (green) environment on mental health and neurodegenerative diseases, b) the glymphatic system in ageing and disease and c) the analysis of data in big international datasets such as UK biobank.

d. Results (4000 characters)

Astrocytes-neurons structural crosstalk
CC analyzed a high-resolution FIB-SEM dataset of 6 dense-reconstructed mice brains, to study the relationship between astrocytic glycogen and neuronal mitochondria, using visual computing approaches, in coll. with M. Agus (HBKU, Qatar) and H. Pfister (Harvard). We found that structural synaptic alterations during aging can be correlated with metabolic alterations visible with unbalanced glycogen load in astrocytes.
CC is developing a metaverse application that allows multiple users wearing VR goggles to access a common space where 3D reconstructions can be visualized, manipulated and analyzed in a collaborative way.
Altered cellular/molecular mechanisms in SMA and therapeutic approaches
AV, MB & RS (with GM) are studying the cerebral cortex of SMA mice at different postnatal ages: compared to WT, we observed i) reduction of both corticospinal and callosal neurons, ii) decrease of GABAergic signal and iii) morphological alterations in parvalbumin interneurons. Moreover, at the spinal cord level, we further studied the impairment of the glycinergic degradation system: by RNA-Scope ISH, AC showed a significant Gcsh increase in SMA MNs, also confirmed in *Smn*-siRNA silenced NSC34 cells. AV and MB are also testing different therapeutic approaches. With AC and RS, we demonstrated that MR-409, a GHRH agonist, is able to reduce muscle atrophy and promote maturation of NMJs, upregulating myogenic genes and inhibiting proteolytic pathways. We are also testing repositioned SMN-dependent (in coll. with Univ. Valencia/Inserm; with GM) and -independent drugs (in coll. with CNR, Naples; with DMR, EN, SS), demonstrating their efficacy in counteracting neurodegeneration, neuroinflammation and/or muscular atrophy.
Stem cell therapy in ALS
MB (in coll. with A. Vescovi; with DMR and CF) is testing the therapeutic potential of clinical-grade human neural stem cells in SOD1 mice: the treatment can slightly delay the decline of motor performances, although upper MN survival and neuroinflammation are not significantly modified. Other targets are under evaluation.

Spinal cord injury

In coll. with Prof. Tonda-Turo (Polytechnic of Turin), MB (with VC) is developing a 3D in vitro spinal cord model by bioprinting technique, to preliminarily screen treatments for SCI. After defined the most suitable hydrogel, the printed construct exhibited well-defined geometry and macroporosity. In vitro assays demonstrated bioink cytocompatibility and revealed that the printing process does not affect cell viability and behavior.

Lifestyle & Active and Healthy Ageing

AV, MB, RS (with GP and SD) are investigating the effect of terpenes exposure i) on mental health affected patients and ii) on in vitro models of Alzheimer disease: preliminary results respectively showed a restoration of some interleukins and miRNAs, and the ability to counteract neurodegeneration and glial activation. We are also setting up hiPSC-derived glutamatergic neurons.

In coll. with Pharmafox, AV, MB, RS (with SD and PP) are testing the effect of several compounds on the stability of the NMJs in sarcopenia in vitro/vivo models.

MB&DMR are evaluating how a stressful lifestyle can impact on ALS onset/progression in disease-predisposed conditions: in vitro results suggest that MNs expressing hSOD1 G93A gene are less able to counteract stress conditions, compared to WT. We are also studying how stress modulates the expression of ALS-related genes.

Investigation of Importin alpha3 (IMPA3) mutant mice in neuronal regeneration, sex and ageing

In coll. with F. Rother (MDC, Berlin), LM (with AP and LT) is studying the role of IMPA3 in sensory and motor behavioral during age sex-based differences. We measure neurite outgrowth from embryos to adult DRG sensory neurons in response to neurotrophins or injury in IMPA3 mutant mice in vitro.

Covid-19 and CNS

We are interested in studying the Covid-19 effects on CNS, by reproducing a cytokine storm on iPSC-derived glutamatergic neurons: we are evaluating early cellular changes, including activation of inflammation-related molecular cascades.

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 2023, we have obtained significant results in the field of SMA and MN diseases, by identifying new drugs which extend the lifespan of the animal models of disease. In particular, the identification of an agonist of GHRH as a protective molecule and of new molecules preventing muscular atrophy by acting on the NMJ 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. We are involved in the technical revolution about microscopy to investigate micro- and meso-connectome, with the use of confocal, light-sheet and 3D-EM microscopy.

f. Publications

1. 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. Brief Bioinform. 24(1):bbac491.
Research article - Q1

2. Alber S, Di Matteo P, Zdradzinski MD, Dalla Costa I, Medzihradszky KF, Kawaguchi R, Di Pizio A, Freund P, Panayotis N, Marvaldi L, Doron-Mandel E, Okladnikov N, Rishal I, Nevo R, Coppola G, Lee SJ, Sahoo PK, Burlingame AL, Twiss JL, Fainzilber M (2023) PTBP1 regulates injury responses and sensory pathways in adult peripheral neurons. Science Advances 9 (30):eadi0286.
Research article - Q1

3. Boido M, Gesmundo I, Caretto A, Pedrolli F, Schellino R, Leone S, Cai R, Sha W, Ghigo E, Schally AV, Vercelli A, Granata R (2023) Agonist of growth hormone-releasing hormone improves the disease features of spinal muscular atrophy mice. Proc Natl Acad Sci U S A. 10;120(2):e2216814120.
Research article - Q1

4. Brasso C, Stanziano M, Bosco FM, Morese R, Valentini MC, Vercelli A, Rocca P (2023) Alteration of the Functional Connectivity of the Cortical Areas Characterized by the Presence of Von Economo Neurons in Schizophrenia, a Pilot Study. J Clin Med. 9;12(4):1377
Research article – Q1

5. Di Pizio A, Marvaldi L, Birling MC, Okladnikov N, Dupuis L, Fainzilber M, Rishal I (2023) A conditional null allele of Dync1h1 enables targeted analyses of dynein roles in neuronal length sensing. Journal of Cell Science 136(5):jcs260220.
Research article - Q1

6. Galbiati M, Meroni M, Boido M, Cescon M, Rusmini P, Crippa V, Cristofani R, Piccolella M, Ferrari V, Tedesco B, Casarotto E, Chierichetti M, Cozzi M, Mina F, Cicardi ME, Pedretti S, Mitro N, Caretto A, Risè P, Sala A, Lieberman AP, Bonaldo P, Pennuto M, Vercelli A, Poletti A (2023) Bicalutamide and Trehalose Ameliorate Spinal and Bulbar Muscular Atrophy Pathology in Mice. Neurotherapeutics 20(2):524-545.
Research article - Q1

7. Menduti G, Boido M. Recent Advances in High-Content Imaging and Analysis in iPSC-Based Modelling of Neurodegenerative Diseases. Int J Mol Sci. 2023 Sep 28;24(19):14689.
Review - Q1

8. Petrelli F, Zehnder T, Laugeray A, Mondoloni S, Calì C, Pucci L, Molinero Perez A, Bondiolotti BM, De Oliveira Figueiredo E, Dallerac G, Déglon N, Giros B, Magrassi L, Mothet JP, Mameli M, Simmler LD, Bezzi P (2023) Disruption of Astrocyte-Dependent Dopamine Control in the Developing Medial Prefrontal Cortex Leads to Excessive Grooming in Mice. Biol Psychiatry 93(11):966-975.
Research article - Q1

9. Ricci FS, Stanga S, Mezzanotte M, Marinaccio C, D'Alessandro R, Somà A, Sottemano S, Conio A, Morana G, Spada M, Boido M, Mongini TE (2023) Biochemical characterization on muscle tissue of a novel biallelic ACO2 mutation in an infant with progressive encephalopathy. JIMD Reports 65(1):3-9.
Research article - Q3

10. Schellino R, Besusso D, Parolisi R, Gómez-González GB, Dallere S, Scaramuzza L, Ribodino M, Campus I, Conforti P, Parmar M, Boido M, Cattaneo E, Buffo A (2023) hESC-derived striatal progenitors grafted into a Huntington's disease rat model support long-term functional motor recovery by differentiating, self-organizing and connecting into the lesioned striatum. Stem Cell Res Ther. 28;14(1):189.
Research article - Q1

11. Schellino R, Boido M, Vrijbloed JW, Fariello RG, Vercelli A (2023) Synergistically Acting on Myostatin and Agrin Pathways Increases Neuromuscular Junction Stability and Endurance in Old Mice. Aging Dis.
Research article - Q1

12. Tassan Mazzocco M, Murtaj V, Martins D, Schellino R, Coliva A, Toninelli E, Vercelli A, Turkheimer F, Belloli S, Moresco RM (2023) Exploring the neuroprotective effects of Montelukast on brain

inflammation and metabolic connectivity in a rat model of quinolinic acid-induced striatal neurotoxicity. J Neuroinflammation 20, 34.
Research article - Q1

13. Traldi C, Chiappini V, Menduti G, Tonda-Turo C, Boido M (2023) Advanced materials and biofabrication technologies to design in vitro functional central nervous system models. Front. Med. Eng. 1:1270943.
Review – na

14. Vercelli A (2023) Turin as a neuroscience cradle. Lancet Neurol. 22(5):381.
Book review – Q1

15. Viana JF, Machado JL, Abreu DS, Veiga A, Barsanti S, Tavares G, Martins M, Sardinha VM, Guerra-Gomes S, Domingos C, Pauletti A, Wahis J, Liu C, Calì C, Henneberger C, Holt MG, Oliveira JF (2023) Astrocyte structural heterogeneity in the mouse hippocampus. Glia 71(7):1667-1682.
Research article - Q1

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 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) in vitro models of neurodegenerative diseases (iPSCs and organoids). We are investigating how urban green environmental spaces and natural compound inhalation affect mental health and can counteract neurodegeneration. We aim at identifying some new therapeutic targets for neurodegenerative diseases (as SMA, ALS and HD). By investigating the role of mitochondrial-iron metabolism in healthy aging and 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.
AV is coordinating the UNITO units involved in the PNRR project D34H, which aims to build a digital and biological twin of the patient, by analysing clinical datasets to predict the evolution of a disease, and another PNRR project, INNOVA, aimed at identifying biomarkers of disease. Meanwhile, we are creating 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.
We are exploiting new techniques for *in vitro* analysis of brain development and disease modeling, such as light sheet microscopy and innovative clearing protocols, bioprinting, 3D EM, and semi-automated tool for 3D analysis in VR.

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, F. Pizzagalli 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 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 cell death in neurodegenerative diseases. We are 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 promote healthy aging and to develop new therapeutic strategies to prevent neurodegenerative diseases and to support brain repair. Therefore, 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

MB is developing a 3D in vitro spinal cord model by bioprinting technique to preliminarily screen treatments for SCI: she will implement the model, by including additional cell types and including a "skeleton" of aligned fibers in the printed model to favor the growth of aligned axons.

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&AV (with AC) will further elucidate the glycinergic system impairment in SMA, by studying glycine neurochemistry and metabolism in SMA mice. With RS, we are studying the remodeling in cortical cytoarchitecture and the possible impaired corticogenesis, due to the lack of SMN, with the aim to better unravel whether morpho-functional alterations in the cortex contribute to disease progression. With GM, MB will extend our studies on GABA signaling and interneuron functionality, by investigating in depth inhibitory synapse density and performing electrophysiological experiments.

Moreover, MB (with GM, ES, DMR, SS) will further test both SMN-dependent and -independent approaches for SMA, testing in vitro and in vivo repurposed drugs, also planning RNA-sequencing experiments to unravel the mechanism of action.

ALS: MB (and DMR) will deepen the ongoing studies, by evaluating the ALS-related pathways affected by stress in human iPSCs-derived motor neurons (hiPSC-MNs) an in vivo.

SMA/ALS: SS (and MM, JCG) will study mitochondrial mobility and function by targeting mitochondrial enzymes and kinases in hiPSC-MNs.

Aging and Alzheimer's disease (AD)

SS (and MM, JCG) by using cellular and animal models of healthy aging and AD will evaluate the levels of the iron pool available for intracellular metabolic reactions in the brain, their possible implications in determining cytotoxic effects, the decline in cognitive and motor skills and how mitochondria, iron and amyloid deposits interact in the brain. Moreover, the effects of long-term regular running exercise on brain iron homeostasis in mice will be investigated during healthy aging in order to verify the beneficial effects of exercise on brain iron overload, trafficking, and consequently on improving cognitive abilities.

AV, MB, RS (with PP and SD), in coll. with Pharmafox Therapeutics AG, will further test molecules for supporting muscle innervation in elderly: to complete the characterization of ActR-Fc-nLG3, we will evaluate its biodistribution and pharmacokinetics.

Mental health and urban green

In coll. 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 modulated by gender, aging, social interaction and rare disease in the peripheral nervous system. Research into this interesting interaction will unlock novel approaches to personalized pain therapy.

iPSCs and organoids

AV and MB (with EN, CF, GP and SV) plan to exploit iPSCs, organoids and assembloids to identify early cellular changes and alterations, that could represent valid and predictive biomarkers, and to preliminarily screen drugs in the perspective of an increasingly effective precision medicine.

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 the Polytechnic and INRIM (Istituto Nazionale di Ricerca Metrologica) will allow to design biosensors and lab-on-chip to the detection of biomarkers. AV&MB will develop in vitro models of pathologies of the nervous system and tumors based on human iPSCs and organoids/assembloids ("biological twin"). We will study these 3D models by a multimodal correlative imaging approach, exploiting different microscopes to apply a multi-scale imaging approach that will traverse mm-scale live-cell light microscopy to nm-scale volume electron microscopy

SS (and MM, JCG) will combine advanced *in vitro* and *ex vivo* techniques to study mitochondrial dynamics during aging and dementia: 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.

We are using iDISCO clearing protocols and light sheet microscopy to generate 3D volume visualizations of whole brains, showing 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), as well as structural astrocytes/neurons interplay (CC). Moreover, a step further we intend to set up is the ExM protocol, to further improve resolution, as intermediate step before 3D EM. We have also recently installed the Zeiss Volutome, the most advanced Serial-Block Face Scanning Electron Microscope (SBF-SEM), that will allow correlative light-electron microscopy approaches to analyze nervous system samples. All these

techniques will require development of novel visualization and analysis techniques that will be developed using the aid of VR.
The collaboration with prof. Cauda (Psychology Dept.) will allow using voxel-based morphometry, fMRI and tractography in ageing subjects. A collaboration is also under discussion to develop a neuroinformatic approach in studies of neurodegenerative diseases with F. Di Cunto, F. Pizzagalli and P. Provero.

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2023

Laboratory name: Clinical Neurobiology

1. LABORATORY DESCRIPTION – PERSONNEL:

Principal Investigator (acting)

1) Di Sapio, Alessia
   Position: Head of the Regional Reference Center for Multiple Sclerosis (CRESM)
   Degree: MD
   Birthdate: 22/10/1966
   Phone: 011 9026 697
   Email: adisapio2210@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 (until 30/06/2023)
   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 (until 30/11/2023)
   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

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) Mohamed Abdel Azim, Gada (since 27/11/2023)
Position: Biologist
Degree: MSc
Birthdate: 30/12/1991
Phone: 011 670 66 35
Email: adam792013@gmail.com
Role & expertise: sample processing and storage in CRESM Biobank, diagnostic tests for MS patients

8) 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	Funding Program/Agency	Role of the unit	Overall Amount Funded	Managed by FCO/UNITO
2022-2023	Convezione con la Fondazione Cavalieri Ottolenghi per la realizzazione di alcune attività del protocollo di ricerca: Biomarcatori di Neurodegenerazione nei pazienti con Sclerosi Multipla e NMOSD: studio pilota di applicazione del dosaggio dei neurofilamenti sierici nella pratica clinica corrente	Marco Capobianco - Alessia Di Sapio	AOU San Luigi Gonzaga	PI	90,000	FCO
2023 - 2025	Ruolo dei NFL nella diagnosi e monitoraggio dei pazienti con Sclerosi Multipla: il progetto pilota del CRESM	Alessia Di Sapio	Roche	PI	100,000	FCO
2022 - 2023	Neurofilamenti liquorali e fattori di evoluzione della Sclerosi Multipla	Marco Capobianco – Alessia Di Sapio	Università di Catania	PI	30,000	FCO

3. SCIENTIFIC ACTIVITIES IN 2023

Alessia Di Sapio (acting PI)

Supervised PhD students:	NA
Honors, prizes, awards:	Winner grant "Bando Roche per i Servizi a supporto di soluzioni innovative per la Sclerosi Multipla", award received April 12, 2023
Outreach activities	
• International collaborations:	Prof. David Leppert, Department of Biomedicine, Basel (Schweiz), project: "Granulocyte activation markers as biomarkers in the differential diagnosis of NMOSD and MOGAD vs MS: a novel diagnostic tool to support timely acute phase therapy"
• Invited talks:	Speaker "La sicurezza del farmaco nella Real Life", Congress "Sclerosi Multipla: si può parlare di neuroprotezione?" IRCCS San Raffaele, Milano, April 5, 2023 Speaker in the "Screen&Care Amiloidosi hATTR " Educational Project Tutorship in the theoretical-practical course "Research methodology and statistics applied to the study of Multiple Sclerosis" Roma, November 24, 2023
• Science communication:	Speaker of the webinar "Stili di Vita e Sclerosi Multipla", February 7, 2023 Speaker of the webinar "La sicurezza dei farmaci", as part of World Patient Safety Day, September 13, 2023 Speaker of the webinar "La Biobanca del CRESM a servizio della ricerca sulla sclerosi multipla", as part of the European Biotech Week, September 27, 2023 Invited Talk "SM e la Ricerca", Congress "Sguardi: #GiovanioltrelaSM, Turin, September 23, 2023
• Editorial duties:	Scientific editorial coordinator in "Siponimod nella sclerosi multipla secondariamnete progressiva con attività di malattia: esperienze di real life", vol n°01
• others	Presenting author for the poster (Abstract ID: 462) titled "*BB-CRESM: a structured institutional biobank for quality research in Multiple Sclerosis*" at the 20th national Congress of the Italian Society for Neuroscience (SINS), Turin, September 14-17, 2023.

	Participation in the "Biobanking" Regional Working Group of DAIRI (Dipartimento Attività integrata Innovazione e Ricerca, Azienda Ospedaliera Alessandria)
Organizational activities and responsibilities at NICO:	NA
Speakers invited:	NA
Other organizational activities:	NA
Workshops, Schools or Conferences organized:	Scientific Director in the "Open Debate: Tailor Therapy and sequencing, Turin, March 7 , 2023 Nord Ovest Network Meeting. Neurodegenerazione e progressione clinica in SM: nuovi segnali predittivi, nuove basi per un approccio comune, Turin, March 10, 2023 Tutor in the Improvement team Meeting "SM debates", Turin April 2nd , 2023 Tutor in the Improvement team Meetings "Improve 2023", Turin, March 3 and June 14 2023 Scientific Responsible for the Residential Event "The role of induction in HET (High Efficacy Treatment), Turin, September 14, 2023
Technology transfer achievements (patents, etc.):	NA

Arianna Sala, Resident Biologist

Supervised PhD students:	NA
Honors, prizes, awards:	NA
Outreach activities	
• International collaborations:	NA
• Invited talks:	NA
• Science communication:	"Sclerosi multipla: diagnosi e terapia", Santorre di Santarosa High School, November 2nd, 2023
• 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

Paola Valentino, Medical Biotechnologist

Supervised PhD students:	NA
Honors, prizes, awards:	NA
Outreach activities	
• International collaborations:	NA
• Invited talks:	Speaker at the conference: “Biobanking e salute pubblica: nuovi scenari”, (Presentation: "Biobanking al servizio della ricerca in Sclerosi Multipla: l'esperienza della Biobanca del CRESM.", 23/06/2023, 4 h, DAIRI_Dipartimento Attività integrata Innovazione e Ricerca Azienza Ospedaliera Alessandria, 1 ECM) Speaker at the conference: “MS Nord-ovest network meeting. Neurodegenerazione e progressione clinica in SM: nuovi segnali predittivi, nuove basi per un approccio comune.”, (Presentation: “Il dosaggio dei Neurofilamenti nella pratica clinica nei pazienti con SM e NMOSD”, 10/03/2023, 8h, BIOMEDIA - Via Libero Temolo 4, 20126 Milano, 7ECM)
• Science communication:	NA
• Editorial duties:	NA
• others	Participation in the ELSI Working Group “Biobancaggio/Biobanche di ricerca e interazione con il garante” Participation in the “Biobanking” Regional Working Group of DAIRI (Dipartimento Attività integrata Innovazione e Ricerca, Azienza Ospedaliera Alessandria)
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

Francesca Montarolo, PhD Biologist

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

• Editorial duties:	Topic Editors at Frontiers in Cellular Neuroscience, Research topic "*The cerebellar involvement in non-cerebellar pathologies",* published Jun 13, 2023.
• others	Presenting author for the poster (092/1) titled "*Fgf14 deletion confers resilience to basal and stress-induced depressive-like behavior* at the 20th national Congress of the Italian Society for Neuroscience (SINS), Turin, September 14-17, 2023. Referee for the grant agency National Science Center Poland (NCN) (OPUS-24). Referee for the grant agency Fondazione Italiana Sclerosi Multipla (FISM).
Organizational activities and responsibilities at NICO:	Host in the NICO Webinar March 24, 2023 invited speaker Dr. Fiorenza Stagni (Università degli Studi di Bologna). Title of presentation "*Potential of early pharmacotherapies for the improvement of intellectual disability in Down syndrome: lesson from the Ts65Dn mouse model".*
Speakers invited:	NA
Other organizational activities:	NA
Workshops, Schools or Conferences organized:	Member of the local organizing committee of the 20th National Congress of the Italian Society for Neuroscience (SINS), Turin, September 14-17, 2023.
Technology transfer achievements (patents, etc.):	NA

Serena Martire, Biostatistician

Supervised PhD students:	NA
Honors, prizes, awards:	NA
Outreach activities	
• International collaborations:	NA
• Invited talks:	Speaker of the course: "Il trapianto autologo di cellule staminali Emolinfopoietiche nella sclerosi multipla (aHSCT)", asynchronous DL organized by Fondazione Italiana Sclerosi Multipla (FISM)
• Science communication:	Speaker of the webinar "La Biobanca del CRESM a servizio della ricerca sulla sclerosi multipla", as part of the European Biotech Week, September 27, 2023
• Editorial duties:	NA
• others	Participation in the "Biobanking" Regional Working Group of DAIRI (Dipartimento Attività integrata Innovazione e Ricerca, Azienda Ospedaliera Alessandria) Member of the Trial Steering Committee of the

	NET-MS study, an Italian multicenter randomized clinical trial aiming at evaluating the use of AHSCT in aggressive multiple sclerosis versus the best available therapy, funded by Fondazione Italiana Sclerosi Multipla (FISM)
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:	NA
• Science communication:	NA
• Editorial duties:	NA
• others	Presenting author for poster titled *"Applicability of sNFL in Multiple Sclerosis as additional monito ring tool in clinical practice and implications in NEDA-3 evaluation"* at the 20th National Congress of the Italian Society for Neuroscience (SINS 2023), Turin, September 14-17, 2023. Presenting author for poster titled *"Comparison between available technologies and biological approaches for CSF and serum NFL quantification: a further step towards implementation in clinical practice"* at the 9th Joint ECTRIMS-ACTRIMS Meeting (MSMilan2023), Milan, October 11-13, 2023.
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:	NA
• Editorial duties:	NA
• others	NA
Organizational activities and responsibilities at NICO:	NA
Speakers invited:	NA
Other organizational activities:	NA
Workshops, Schools or Conferences organized:	NA
Technology transfer achievements (patents, etc.):	NA

Gada Mohamed Abdel Azim, Biologist

Supervised PhD students:	NA
Honors, prizes, awards:	NA
Outreach activities	
• International collaborations:	NA
• Invited talks:	NA
• Science communication:	NA
• Editorial duties:	NA
• others	NA
Organizational activities and 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, MD

Supervised PhD students:	NA
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	
• Invited talks:	Speaker at the conference “All’orizzonte e ai confini della neurologia”, (Presentation: “Rallentare, congelare, guarire la Sclerosi multipla”), Cuneo, Februrary 10, 2023 Speaker at the conference “SMisura”, Milan, December 1st, 2023

• Science communication:	
• Editorial duties:	Editor in chief for Neurology and Therapy
• others	Member of the Organizing Committee for Continuing Education, Associazione Italiana Sclerosi Multipla (AISM)
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 2023

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.

After purchasing the SR-X instrument (Quanterix) for sNFL quantification in 2018, we defined specific decade-related cut-off values to discriminate high pathological from normal sNFL values and we evaluated sNFL in different clinical contexts. Recently, we applied sNFL dosing in the monitoring of MS patients treated with Natalizumab, one of the most effective treatment options. However, its use is associated to the development of progressive multifocal leukoencephalopathy (PML), caused by JC virus. The occurrence of PML causes the death in 20% of cases and serious disability in 40% of survivors. Patients monitoring is crucial to early recognize PML signs and symptoms, and guidelines indicate to perform frequent brain MRI for the duration of the treatment. In this context, additional sensitive biomarkers reflecting clinical signs and brain lesions are needed for the early detection of PML onset. Some few studies have demonstrated that sNFL levels are strongly elevated at PML diagnosis and correlate with MRI lesions' volume; still, data analysis at individual level are lacking, limiting a simple and correct interpretation of results in single patients.

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) assessing the ability of sNFL to early detect PML in longitudinal patients' follow up.
II) 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) we retrospectively measured longitudinal sNFL in the four PML cases diagnosed in our MS center between 2009 and 2015, which were monitored by MRI and clinical parameters. Further, sNFL were tested in a group of NTZ-treated patients experiencing NEDA-3 status for at least 12 months, as controls. All samples were provided by the BB-CRESM. sNFL levels were interpreted according to previously defined reference values. Elevated sNFL were observed not only at PML diagnosis, but also in pre-PML phase. At PML recovery, sNFL weren't normalized in all patients' samples, suggesting ongoing neuronal degeneration.
II) Currently, samples from 11705 blood drawn are collected in BB-CRESM. In year 2023, samples from 1271 blood drawn were collected. BB-CRESM has been contacted and involved in several collaborations with public and private research institutions and pharma-companies. In 2023, 70 samples and associated data have been distributed. BB-CRESM 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 indicate that sNFL represent a reliable biomarker and should be introduced in clinical practice as an additional/alternative parameter to MRI to early detect and monitor PML. 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

1. Vecchio D, Puricelli C, Malucchi S, Virgilio E, Martire S, Perga S, Passarelli F, Valentino P, Di Sapio A, Cantello R, Dianzani U, Comi C (2023). Serum and cerebrospinal fluid neurofilament light chains measured by SIMOA™, Ella™, and Lumipulse™ in multiple sclerosis naïve patients. Multiple Sclerosis and Related Disorders, 105412.
Research article – Q1

2. Iaffaldano P, Lucisano G, Guerra T, Patti F, Cocco E, De Luca G, Brescia Morra V, Pozzilli C, Zaffaroni M, Ferraro D, Gasperini C, Salemi G, Bergamaschi R, Lus G, Inglese M, Romano S, Bellantonio P, Di Monte E, Maniscalco GT, Conte A, Lugaresi A, Vianello M, Torri Clerici VLA, Di Sapio A, Pesci I, Granella F, Totaro R, Marfia GA, Danni MC, Cavalla P, Valentino P, Aguglia U, Montepietra S, Ferraro E, Protti A, Spitaleri D, Avolio C, De Riz M, Maimone D, Cavaletti G, Gazzola P, Tedeschi G, Sessa M, Rovaris M, Di Palma F, Gatto M, Cargnelutti D, De Robertis F, Logullo FO, Rini A, Meucci G, Ardito B, Banfi P, Nasuelli D, Paolicelli D, Rocca MA, Portaccio E, Chisari CG, Fenu G,

Onofrj M, Carotenuto A, Ruggieri S, Tortorella C, Ragonese P, Nica M, Amato MP, Filippi M, Trojano M; Italian MS Register (2023). Evaluation of drivers of treatment switch in relapsing multiple sclerosis: a study from the Italian MS Registry. J Neurol. 10.1007/s00415-023-12137-8.
Research article – Q1

3. Valentino P, Malucchi S, Bava CI, Martire S, Capobianco M, Malentacchi M, Sperli F, Oggero A, Di Sapio A, Bertolotto A. Serum Neurofilaments are a reliable biomarker to early detect PML in Multiple Sclerosis patients (2023). Mult Scler Relat Disord. 77:104893.
Research article – Q1

4. Toscano S, Oteri V, Chisari CG, Finocchiaro C, Lo Fermo S, Valentino P, Bertolotto A, Zappia M, Patti F. Cerebrospinal fluid neurofilament light chains predicts early disease-activity in Multiple Sclerosis (2023). Mult Scler Relat Disord. 80:105131.
Research article – Q1

5. Bonaldo B, Casile A, Montarolo F, Bertolotto A. Modeling Multiple Sclerosis in the Two Sexes: MOG35-55-Induced Experimental Autoimmune Encephalomyelitis (2023). J Vis Exp. (200).
Research article – Q2

6. Giordano A, Testa S, Bassi M, Cilia S, Bertolotto A, Quartuccio ME, Pietrolongo E, Falautano M, Grobberio M, Niccolai C, Allegri B, Viterbo RG, Confalonieri P, Giovannetti AM, Cocco E, Grasso MG, Lugaresi A, Ferriani E, Nocentini U, Zaffaroni M, De Livera A, Jelinek G, Solari A, Rosato R. Applying multidimensional computerized adaptive testing to the MSQOL-54: a simulation study (2023). Health Qual Life Outcomes. 21(1):61.
Research article – Q1

7. Bruschi N, Malentacchi M, Malucchi S, Sperli F, Martire S, Sala A, Valentino P, Bertolotto A, Pautasso M, Capobianco MA. Tailoring Rituximab According to CD27-Positive B-Cell versus CD19-Positive B-Cell Monitoring in Neuromyelitis Optica Spectrum Disorder and MOG-Associated Disease: Results from a Single-Center Study (2023). Neurol Ther. 12(4):1375-1383.
Research article – Q2

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):

Based on the results obtained in the last year and during more than a decade of research activity at NICO, 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 funding from Fondazione Italiana Sclerosi Multipla (FISM) and 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. To achieve this goal we applied to the annual call for proposals by Fondazione Italiana Sclerosi Multipla (FISM).
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. This aim will be realized thanks to the sponsorship by Roche.
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. This aim will be realized thanks to the sponsorship by Roche.
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 assessedby Simoa technology on SR-X instrument including GFAP, TAU, Ab42, Ab40, alpha-sinuclein. In addition, the technology enables to set-up custom assays, when specific antibodies are available for the analyte of interest.

NeuroWebinar & Seminar 2023
one appointment/week, sually uon Friday at 2.00 pm
**Hybrid seminar*: both in presence and on webex*

Friday 20/1/23 - Hybrid seminar

Tommaso Pizzorusso, BIO@SNS laboratory, Scuola Normale Superiore of Pisa; Institute of Neuroscience CNR, Pisa

Genetic and environmental regulation of visual cortical plasticity

The visual cortex is characterized by developmental periods of high plasticity designated critical periods. However, environmental factors are able to modulate plasticity levels also in adult animals. Indeed, exposing animals to an enriched environment (EE) has dramatic effects on brain structure, function, and plasticity also in adult animals. The poorly known ‘‘EE-derived signals’’ mediating the EE effects are thought to be generated within the central nervous system. In the talk I will report data about intrinsic regulators of cortical plasticity and the interaction with signals originating from the periphery that can be changed by life style. The role of the gut microbiota and the effect of diet will be discussed

Host: Serena Bovetti

Friday 27/1/23 - Hybrid seminar

Indrek Koppel, Tallinn University of Technology, Estonia

Cell type-specific omics of neurons and glia

For studying transcriptomes in a cell type-specific manner researchers can choose between single-cell strategies and affinity purification of ribosome-associated mRNA. Single cell proteomics is in very early stages of development, but cell-specific labeling and affinity purification of proteins has been achieved using genetic tools, ensuring incorporation of affinity tags in cell types of interest. In this talk, I will discuss currently available tools in cell type-specific proteomics and introduce a method that leverages a puromycin inactivating enzyme for achieving cell type specificity. I will describe the use of this strategy for studying protein synthesis in co-cultures of rat cortex cells. In addition, I will talk about our efforts in studying neuron-astrocyte communication using tools for cell-specific activation and translatomics.

Host: Letizia Marvaldi

Friday 10/2/23 - Hybrid seminar

Samuele Negro, Department of Biomedical Sciences, University of Padova IT

CXCR4: a new target to boost peripheral nerve regeneration

The peripheral nervous system (PNS) is the part of the nervous system outside the brain and spinal cord that relays signals between the central nervous system (CNS) and the rest of the body. It is composed mainly of: 1) neuronal cells, in particular a combination of motor, sensory and autonomic neurons; 2)

Schwann cells (SCs), glial cells which ensheaths nerves in a layer of myelin and provide trophic support; 3) other non-neuronal cells such as fibroblast and satellite cells. Despite the PNS has an intrinsic ability for repair and regeneration to a certain extent, differently from the CNS, peripheral nerve injuries represent an important clinical issue with insufficient or unsatisfactory therapeutic approaches.
The process of nerve regeneration is complex, involving many factors concerning to the neuron and the cellular environment, and is still far from being understood. Our research group has contributed to shedding light on some of the mechanisms that govern PNS regeneration. In particular, we have recently discovered that the molecular axis orchestrated by the chemokine CXCL12α and its receptor CXCR4 is a novel signalling pathway that powerfully stimulates peripheral nerve regeneration.

Host: Roberta Schellino

Friday 3/3/23 - Webinar

Wenhui Huang, Universität des Saarlandes Homburg, Germany

Adenosine control of glial fate and functions

Extracellular adenosine is mainly formed from the sequential ATP metabolism by a series of hydrolases, in which ecto-5'-nucleotidase (Nt5e, also known as CD73) performs the last step converting AMP to adenosine. Under cellular stress conditions, such as inflammation, hypoxia, etc., extracellular adenosine can be drastically increased. In the CNS, adenosine serves as a neuromodulator by triggering various G-protein-coupled adenosine receptors (ARs) of the A1, A2a, A2b and A3 subtypes among which A1 receptors (A1ARs), coupled to $G_{i/o}$ proteins, are the most abundant subtype. A1ARs are widely expressed in the brain, including neurons, microglia, astrocytes, and oligodendrocyte (OL) lineage cells. Transcriptomic studies revealed the highest A1AR expression levels in OL lineage cells and astrocytes, indicating an important role of adenosine signaling in regulating the fate and functions of these glial cells. In my talk, I will introduce our ongoing work studying the *in vivo* functions of adenosine signaling in glial cells by analysing cell type-specific A1AR deficient mice in a cuprizone-induced de-/remyelination model as well as in a peripheral lipopolysaccharide (LPS) injection model.

Host: Enrica Boda

Friday 10/3/23 - Hybrid seminar

Paolo Giacobini, INSERM Research Director, Lille Neuroscience & Cognition Development and Plasticity of the Neuroendocrine Brain

The scent-sational role of GnRH neurons

Gonadotropin-releasing hormones (GnRH) neurons are the master regulators of fertility in vertebrates. Hypothalamic GnRH-secreting neurons release their hormone through the median eminence (ME) into the hypophyseal portal system to stimulate the production and release of pituitary gonadotropins, which regulate the development and function of the gonads. To ensure reproductive success, GnRH neurons have to process and integrate various internal and external cues to elicit the most adapted neuroendocrine responses.
We recently identified, both in mice and humans, a sub-population of extra-hypothalamic GnRH neurons located in the olfactory bulb (OB), whose role has never been investigated. Combining mouse genetics with Cre-dependent viral tracing approaches, and 3D-imaging of whole-mouse heads, we revealed that OB-GnRH neurons project neurites contacting the vomeronasal organ, the chemosensory system that perceives and processes stimuli related to social and reproductive behaviors in many species of vertebrates. In addition, OB-GnRH neurons send long projections to the hypothalamic areas involved in the control of gonadotropin release.
Bidirectional chemogenetic neuromodulation combined with behavioral testing, electrophysiological recording and two-photon *in vivo*calcium-imaging, demonstrated a novel role for this extra-hypothalamic GnRH neuronal population as a central regulatory hub linking pheromonal stimulation with the neuroendocrine response regulating reproduction and mating behavior.

Host: Silvia De Marchis

Thursday 9/3/23 - Hybrid seminar

Philip Greulich, University of Southampton

Mathematical modelling in cell biology: why and when is it useful?

Mathematical and computational modelling has become an increasingly popular tool in the biological sciences. Yet, often modelling is being employed without gaining much value from it, or in ways where

machine learning approaches would be superior. Sadly, wrong use of model fitting can also lead to plainly wrong results. In this talk, I wish to outline some contemporary uses of mathematical modelling in (cell) biology and explain which modelling approaches are generally fruitful and can enrich biological research, in ways that are not achievable by plain experimental approaches.
I will exemplify this on some research projects about clonal dynamics in developing tissue -- mouse mammary gland during pregnancy and microglia in the developing brain -- where experimental data and mathematical modelling were successfully combined to drive the discovery of stem cell fate choice patterns. These examples will show where the pitfalls of mathematical modelling lie and how to circumnavigate them to achieve scientifically sound outcomes that go beyond the reaches of experimental research.

Host: Federico Luzzati

Friday 17/3/23 - Webinar

Elena Choleris, Department of Psychology and Neuroscience Program, University of Guelph, Ontario, Canada

Neuroendocrinology of social cognition in female and male mice

Recent findings demonstrate rapid hormonal facilitation of social cognition in various brain regions of female a male mice. In females, in the Dorsal Hippocampus estradiol's rapid facilitating effects are broad across multiple (but not all tested) learning tasks. In the Paraventricular Nucleus of the Hypothalamus (PVN), Medial Amygdala (MeA) and Medial Prefrontal Cortex, instead, the effects appear specific to social cognition, other types of learning tasks being unaffected. In the PVN and MeA those effects depend upon the action of MeA oxytocin receptors and do not extend to males. In males, in the Bed Nucleus of the Stria Terminalis, estradiol, testosterone and dihydrotestosterone all rapidly facilitate social recognition while inversely affecting object recognition, and those effects depend upon Arginine Vasopressin receptors V1aR in the Lateral Septum. Together, these investigations are highlighting the rapid hormonal regulation of brain networks of females and males subserving social cognition, further advancing our understanding of hormonal regulation of the social brain.

Host: Gruppo Panzica Gotti

Wednesday 22/3/23 - Hybrid Seminar

Christel Genoud, Electron Microscopy Facility, University of Lausanne

The benefits and challenges offered by visualization of large volume with light and scanning Electron Microscopy

In recent years, the field of microscopy has advanced significantly, allowing for the visualization of larger biological structures in 3 dimensions at ever-increasing resolutions. In cellular electron microscopy, this evolution is allowed by the technological developments in volume EM and Correlative multi-modal imaging. These techniques offer a wealth of benefits, including the ability to visualize large volumes of tissue with remarkable detail as well as target rare events in large volumes. However, there are also significant challenges associated with these techniques, including sample preparation, data acquisition, and image processing.
In this talk, we will explore some of the benefits and challenges of these approaches, with a focus on their applications in biological research. We will discuss recent advances in these techniques, including the use of machine learning to aid in data analysis, and highlight some projects that have been made using these methods. Ultimately, we will demonstrate that while these techniques are not without their challenges, they offer tremendous potential for advancing our understanding of the biological world.

Host: Corrado Calì

Friday 24/3/23 - Hybrid Seminar

Fiorenza Stagni, Università degli Studi di Bologna

Potential of early pharmacotherapies for the improvement of intellectual disability in Down syndrome: lesson from the Ts65Dn mouse model

Down syndrome (DS) is a relatively high-incidence genetic condition caused by the triplication of chromosome 21. Gene triplication may compromise different body functions but intellectual disability represents the unavoidable hallmark and the most invalidating aspect of this pathology. Intellectual disability is mainly attributable to neurogenesis and dendritogenesis alterations that can be traced back to fetal life stages. Although the progressive improvement in medical care has led to a notable increase in

life expectancy for people with DS, there are currently no effective therapies for intellectual disability in DS. Since neurodevelopmental defects are present starting from fetal life stages, early pharmacological interventions are likely to represent a good strategy to improve brain development in DS. With this idea in mind, our research group has examined the efficacy of different pharmacotherapies administered during the prenatal or early postnatal period in the Ts65Dn mouse, a model that recapitulates many anatomical and functional alterations of DS.This talk will describe and discuss the most suitable time windows for treatment and some of the attempted pharmacotherapies targeted to pathways that are known to be deranged in DS, that have proved to be effective in restoring trisomy-linked neurodevelopmental defects and cognitive performance in the Ts65Dn mouse model.In view of the good translational impact of some of these tested therapies, our preclinical findings may open the way for clinical trials in individuals with DS, thereby improving their life conditions.

Host: Francesca Montarolo

Friday 7/4/23 - Webinar

Alain Prochiantz, Emeritus Professor at College de France and Chief Scientific Officer BrainEver SAS

OTX2 and EN1 homeoprotein transduction, from physiopathology to therapeutic strategies

Intercellular transfer has now been demonstrated for 150 homeoprotein transcription factors. However, the physiological functions served by this novel signaling pathways have only been studied for a handful of them, including OTX2 and ENGRAILED. The conference will focus on the role of OTX2 and ENGRAILED-1 signaling in the regulation of cerebral cortex plasticity and spinal cord a-Motoneuron physiology, respectively. The consequences of the latter recent findings in the development of original therapeutic strategies in mood disorders and Amyotrophic Lateral Sclerosis will be discussed.

Host: Serena Stanga

Friday 21/4/23 - Hybrid Seminar

Alessandro Ferrarini, Account Manager Starlab

The Sustainable Laboratory

It is universally acknowledged that the Pharmaceutical Industry and Scientific Research sector are highly polluting, in terms of CO2 emissions, plastic waste and water usage. A study conducted in2015 estimated that labs worldwide consume around 5 million tons of plastic and that a research lab requires 5 to 10 times the amount of energy used in an office of the same size.

The current situation is alarming and rapidly deteriorating. The Pharma and Life Science Sector has one of the largest carbon footprints globally, estimated to be even higher than the automotive industry. While the Pharmaceutical and healthcare sectors are clearly the biggest contributors, scientific research also play a role in the overall result, with plastic waste and energy consumption being the most significant factors.

Starlab mission is to continually look for intelligent, climate-friendly products and processes. We try to raise awareness among scientists and present possible solutions: The new TipONE generation saves up to 40% in plastic and the smart gloves packaging optimizes space and transportation. Starlab is constantly thinking about sustainability, with the ultimate goal to become a Green Company in every aspect. So, Let’s get green Together.

Host: Serena Stanga

Friday 19/5/23 - Hybrid Seminar

Silvia Gancheva Marinova e Antoaneta Georgieva (University of Varna, Bulgaria)
Silvia Gancheva Marinova, MD, PhD

Pharmacologically induced changes in osteocalcin levels – metabolic and central nervous system effects in healthy and metabolic rats

Osteocalcin is a bone-derived protein involved in the regulation of energy metabolism and CNS functions in rodents. Its serum concentrations can be modified pharmacologically in opposite directions through administration ofvitamin K antagonists and bisphosphonates. The current presentation describes the resulting changes in energy metabolism and brain functions.

Antoaneta Georgieva, MD, PhD
Effects of Aroniamelanocarpa fruit juice and its component chlorogenic acid on the ovariectomy-induced behavioral changes in rats
Presentation of our research on the behavioral changes in a rat model of ovariectomy-induced estrogen deficit and the effects of a 10-week treatment with *Aroniamelanocarpa*fruit juice in two different doses or chlorogenic acid.
Host: Ilaria Bertocchi/Carola Eva

Tuesday 6/6/23 - Hybrid Seminar

Marco Cambiaghi, University of Verona - Dep. of Neurosciences, Biomedicine and Movement Sciences
Electrified brains: from the torpedo fish to transcranial direct current stimulation
The idea of modulating brain activity with a non-invasive approach has always been one of the major goals of neurophysiology and to a broad extent, of modern neurology and psychiatry, since the associated disorders are often the consequences of dynamic plastic changes of the neural networks. Well before the discovery of the physical phenomenon, electricity was used as a therapeutical tool but only the last few decades saw the development of effective non-invasive neuromodulatory techniques. Among them, transcranial direct current stimulation (tDCS) has recently emerged as a safe and economic tool to guide neuroplasticity and modulate cortical function by tonic stimulation with weak direct currents. Despite its wide use in human studies, some underlying mechanisms of action have been clarified only recently and the vast majority is still to be elucidated. In recent years, we are focusing on the study of indirect effects of tDCS and the influence of brain state during stimulation, in different preclinical models in both physiological and pathological conditions. In particular, we explored prefrontal tDCS influence on dorsal raphe activity and, in the motor cortex, the effects of combining tDCS with physical activity.
Host: Enrica Boda

Friday 9/6/23 - Hybrid Seminar

Marta Valenza, Department of Biosciences, University of Milan
Astrocyte-neuron interplay in the cholesterol dysfunction in Huntington's disease brain: from the mechanism to therapeutics
In the adult brain, neurons require local cholesterol production, which is supplied by astrocytes. Cholesterol biosynthesis is severely reduced in the brain of Huntington's disease (HD), a genetic adult-onset neurodegenerative disease characterized by synaptic dysfunction and motor and cognitive defects. The defect occurs inastrocytes with detrimental consequences on HD neurons' activities. The underlying molecular mechanism is a reduced activity of SREBP2, the transcription factor that activates the expression of many genes involved in cholesterol synthesis.
In the last years, different in vivo strategies were developed to counteract cholesterol dysfunction in HD mouse models either supplying exogenous cholesterol with brain-permeable nanoparticles or enhancing endogenous cholesterol within the HD brain. I will present an overview of thesecholesterol-based strategies and their translational potential. Then, I will focus on the gene therapy approach to force endogenous cholesterol biosynthesis in the striatal astrocytes of HD miceto highlight the relevance of the astrocyte-neuron interplay in HD pathogenesis in vivo.
Host: Marina Boido

Thursday 15/6/23 - Hybrid Seminar

Shimon Ben Shaabat, Department of Biochemistry & Pharmacology, Faculty of Health Sciences, Ben Gurion University, Israel
Role of phytocannabinoids in neuroinflammation and Nanotechnological approach for topical-dermal delivery and intranasal-direct brain targeting
Multiple sclerosis (MS) is a widespread chronic neuroinflammatory and neurodegenerativeDisease. CBG, and CBDA, phytocannabinoids, have attracted significant pharmacological interest due to their non-psychotropic nature. We studied the effects of these compounds on microglial inflammation in vitro, followed by an *in vivo* study. CBG and CBDA attenuated the microglial production of NO in BV2 microglia and primary glial cells and reduced iNOS expression. TNF-a on the other hand was decreased by CBG

but increased by CBDA. The same was found in MS*in vivo*model, experimental autoimmune encephalomyelitis (EAE). The clinical scores of EAE mice were attenuated and lumbar sections of EAE mice showed enhanced neuronal loss. Despite the potential activity, the delivery of phytocannabinoidsto the periphery and to CNS remains challenging. We have developed a new particulate system capable of deliveringphytocannabinoidsinto the periphery by transdermal delivery and to the brain via the intranasal route. In cultures of LPS-induced inflamed BV2 cells, the phytocannabinoid-loaded starch nanoparticles demonstrated low toxicity while effectively reducedNO production and IL-6 levels. Intranasal administration of CBD-loaded starch nanoparticles resulted in higher levels of CBD in the brain than an identically administered CBD solution.

Host: Ilaria Bertocchi and Carola Eva

Friday 23/6/23 - Webinar

Vanessa De Luca, IIT - Genova, Italia

GENDER DIMENSION IN RESEARCH

Horizon Europe is the new European Research & Innovation funding program, following Horizon 2020. Across its commitments, the gender dimension emerges as a cross-cutting objective. Particularly, a set of interventions promises to tackle gender inequalities by dismantling material and cultural barriers that leave women at a disadvantage within the research sector. This talk will focus on conceptual and practical issues concerning the gender dimension in research exploring a few practical examples of how to integrate gender-sensitive contents and methods in research, and apply them when developing new projects and/or funding procedures under the Horizon framework.

Host: Stefano Zucca

Wednesday 28/6/23 - Hybrid Seminar

Hakeem O. Lawal, Delaware Center for Neuroscience Research and Department of Biological Sciences, State University, Dover - Delaware

Drosophila models of Parkinson's Disease and Aging

Drosophila is an excellent model system for the study of many human neurological disorders and states. Parkinson's disease represents one such example. The second most common neurological disease, it is characterized by the loss of dopaminergic neurons of the substantia nigra pars compacta. It has no known cure and current treatments cause severe side effects.

This status quo necessitates the deployment of every model system available to help accelerate progress towards understanding both the cause of the disease and the development of viable treatment strategies. Here we present findings from our lab that demonstrate the utility of Drosophila as a model system to understand the possible underlying causes of this disease and to develop effective treatment strategies. Similarly, we present work from group establishing a role for cholinergic synaptic transmission in the central nervous system on behavioral changes that occur during aging. Moreover, we show that overexpression of the vesicular acetylcholine transporter (VAChT), which mediates the packaging of acetylcholine into synaptic vesicles for exocytotic release, causes a reduction in lifespan and a decline in ACh-linked behaviors during aging. Together, our work adds importantly new light to the contributions of Drosophila as a model for advancing our understanding of both normal and pathological aging.

Host: Ferdinando Di Cunto

Friday 7/7/23 - Hybrid Seminar

Mike Fainzilber, Weizmann Institute of Science, Israel

Neuronal Injury Signaling: SINEs of Growth?

Importins, molecular motors and RNA binding proteins function in a bidirectional mechanism of intracellular communication, consisting of anterograde RNA transport, local translation at axon tips, and retrograde transport of the resulting proteins, for neuron length sensing and growth control. The talk will focus on recent findings revealing an unexpected role for a specific subgroup of non-coding RNAs in this mechanism.

Host: Letizia Marvaldi

Friday 8/9/23 - Webinar

Introduction to BioRender

Host: Letizia Marvaldi

Friday 22/9/23 - Hybrid Seminar

Pascal Hot, Université Mont-Blanc - Chambery

Functional specialisation of the medial temporal lobe and hippocampus: the representational approach

A growing body of research has shown that medial temporal regions play a role outside the memory domain, as they would be specialised in processing certain types of representations. In this view, the hippocampus and perirhinal cortex (PRC) would represent scenes and entities, respectively, independently of the memory or non-memory operation performed on it (Cowell et al., 2019). Using both fMRI (Gardette et al., 2023a, 2023b) and patients with right or left temporal lobectomy, we tested this prediction in the operations of pattern-completion (i.e., the operation involved in recollection), familiarity-based recognition and rejection, and visual discrimination of scene and object images.
We will present our recent data from these studies supporting the representational view of the MTL: the engagement of these regions in processes such as recollection and familiarity would be determined by the representation involved rather than by the operation.

Host: Serena Bovetti

Thursday 12/10/23 - Hybrid Seminar

Takehiro G. Kusakabe, Nanako Okawa, and Ayana Maruo, Konan University, Kobe, Japan

Ascidians: a simple chordate model to study the nervous system

Takehiro G. Kusakabe, PhD

The central nervous system of proto-vertebrate ascidians: a simple but informative model of the vertebrate brain

Vertebrates have evolved the complex brain with sophisticated cranial sensory organs and neuroendocrine systems. Invertebrate chordate ascidiansare the closest living relatives of vertebrates; the clade consisting of tunicates and vertebrates is called Olfactores.By using the ascidian *Ciona intestinalis* type A (also called *Cionarobusta*), we have revealed that ascidians have photoreceptive and gonadotropin-releasing hormone systems, which are similar to those of vertebrates. In this seminar, we introduce conserved and unique features of the ascidian nervous systemand present our recent findingsand evolutionary perspectives.

Nanako Okawa, PhD

Studies on neuron-glia interactions using optogenetics and Ca^{2+}imaging in Ciona larvae

The *Ciona* larva has a central nervous system homologous to that of vertebrates. Its nerve cord, the spinal cord homolog, is mainly composed of glial ependymal cells, along which run axons of cholinergic neurons that reside in the motor ganglion, which is the hind brain homolog. We visualized the activity of glial cells in the nerve cord of *Ciona* larvae by the Ca^{2+}imaging method using G-CaMP8, and we found that active Ca^{2+}transients were associated with tail motions and swimming behavior in response to light. We further analyzed the relationship between neuronal activation and glial cell activity using optogenetics in combination with Ca^{2+}imaging. Our resultsrevealed that the glial ependymal cells in the nerve cord receive cholinergic input from the motor ganglion. Consistently, receptors for various neurotransmitters were shown to be expressed in the glial cells of the nerve cord. Our findings suggest that the glial cells actively interact with neurons and are involved in the control of swimming behavior.

Ayana Maruo, MSc

Spatial transcriptomic analysis of the adult brain of Ciona intestinalis type A

The swimming larvae of ascidians metamorphose into sessile adults, and the adult brain is formed after metamorphosis from a part of the larval brain. In contrast to our detailed knowledge of the larval brain, little is known about the function, cellular composition, and developmental mechanisms of the adult brain. To lay the foundation for elucidating the structure and functions of the adult ascidian brain and its developmental mechanisms, we performed spatial transcriptome analysis of the adult brain of the ascidian *Ciona intestinalis* type A. Our analysispresents, for the first time, comprehensive gene expression data of the ascidian adult brain and gives insights into its structure and function.

Host: Giovanna Gambarotta

Friday 13/10/23 - Webinar

Chrystian Junqueira Alves, Icahn School of Medicine at Mount Sinai - New York

Mechanoregulation of Neurogenesis and Cancer: an Ancient Molecule Controlling Stem Cell Fate and Motility

Plexins are known as axon guidance receptors. However, Plexins originated in unicellular organisms greater than 600 million years ago. Dr. Junqueira Alves' research aims to understand the fundamental role of Plexins during neurogenesis and cancer migration. Using cerebral organoids, he found that Plexin-B2-deficient neuroprogenitors undergo spontaneous neurogenesis. In cancer cells, Plexin-B2 is critical to promote invasiveness. Currently, he is developing novel strategies to accelerate the differentiation of stem cells by nuclear mechanics.
He is also exploring the role of plasma membrane and nucleus mechanoregulation for cancer migration. His research has the potential to accelerate the generation of neurons for disease modeling and discover new mechanisms of cancer migration for drug development.

Host: Roberta Schellino

Friday 20/10/23 - Hybrid Seminar

Francesco Moneta, Preclinical Imaging Division - Bruker BioSpin

Preclinical MRI and PET solutions and applications in neuroscience

Magnetic resonance imaging (MRI) and positron emission tomography (PET) are the core of Bruker portfolio in the field of preclinical research on small rodents. It will be presented the state of the art solutions for preclinical MRI and PET and some examples of their applications in the neurological field

Host: Alessandro Vercelli

Wednesday 25/10/23 - Hybrid Seminar

Ana C. Cristóvão, CICS-UBI Health Sciences Research Centre, University of Beira Interior; NeuroSoV-Fastprinciple-Lda, UBIMedical - Covilhã, Portugal

Ionic-liquid Nox1 inhibitor to be used as a therapeutic solution to delay the progression of Parkinson's disease.

Parkinson's Disease (PD) is a chronic neurodegenerative disorder affecting up to 10 million people worldwide. Despite the efforts to develop a cure for PD, current therapeutic approaches can only target the symptoms. As symptomatic therapies lose effectiveness over time, patients end up with no therapeutic options. Therefore, delaying the disease progression became a promising solution to deal with this disorder.
PD pathogenesis is highly influenced by oxidative stress, and we have previously shown that ROS generated by NADPH oxidase 1 (Nox1) has detrimental impacts on dopaminergic neurons, being a valuable target for therapeutic developments. Inline, we have tested a chemical inhibitor ionic liquid for Nox1 inhibitor (N1inh-IL) capable of preventing neurodegeneration in experimental PD models.
In vitro studies showed that N1inh-IL has no cytotoxic effect on N27 neurons, while it significantly prevents the neurotoxic effect of two specific neurotoxins 6-hydroxydopamine (6OHDA) and 1-methyl-4-phenylpyridinium (MPP+). *In vivo*, the dopaminergic neuroprotective capacity of N1inh-IL, was evaluated in two animal models of PD, one induced by intrastriatal injection of 6OHDA and the other by chronic exposure to paraquat (PQ). The infusion of the N1inh-IL into the right ventricle did not cause neuronal toxicity, while it could prevent 6OHDA-induced neurodegeneration in the *substantia nigra* (SN) of mice. Moreover, four weeks after been exposed to PQ, the brain intraventricle diffusion or the intranasal delivery of N1inh-IL in rats was capable to prevent the motor dysfunction induced by the toxin and the accumulation of alpha-synuclein in the SN.
These results highlight that N1inh-IL can be an innovative therapy to reduce the speed of the progression of PD.

Host: Marina Boido, Serena Bovetti, Serena Stanga

Wednesday 8/11/23 - Hybrid Seminar

Fernando De Castro, Cajal Institute, Spanish National Research Council, Madrid, Spain

Spontaneous remyelination: hot challenge for Multiple Sclerosis and the gate to go beyond

Spontaneous remyelination has been underestimated to date when treating patients with multiple sclerosis. The first results of a clinical trial that opened the door to this major challenge for Neurology were published around this time, 6 years ago. However, many attempts have failed to go further, basically due to weaknesses in the design of preclinical studies that have fueled false hopes. Our group has specialized in improving these preclinical studies and in exploring new compounds that can be incorporated in the future into the therapeutic arsenal with which to treat multiple sclerosis in combination with immunomodulators available in the clinic. We will show some of these cellular and molecular

mechanisms, with special focus on small molecules and aptamers.
Host: Alessandro Vercelli

Thursday 30/11/23 - Hybrid Seminar

Elif Keshinoz, Department of Anatomy, School of Medicine, Acibadem Mehmet Ali Aydinlar University - Istanbul, Turkey.

Mitochondrial alterations in Alzheimer's Disease: Insights from the 3xTg Model

Aim: The 3xTg mouse model mimics key features of Alzheimer's disease (AD) through APP, PS1, and tau mutations. Mitochondrial dysfunction in AD results from interactions between these genes, leading to Aβ and tau deposits, damaging mitochondria, generating ER stress, and resulting in decreased ATP production, impaired neurons, and cell death. Mitochondrial dysfunction also leads to oxidative stress, inflammation, and the formation of amyloid plaques and neurofibrillary tangles, contributing to AD pathology. Mitochondria-ER contact sites (MERCs) are essential for cellular functions, including calcium signaling, lipid metabolism, and molecule exchange across organelles. An alteration may impact cellular calcium regulation, mitochondrial function, and Alzheimer's pathogenesis. The study aims to investigate the role of mitochondrial alteration and MERCs in the 3xTg mice model of AD.
Materials and Methods: In this study, electron microscopic images of brain tissues from the CA3 regions of the hippocampus were taken at 10,000X magnification in 3xTg and Wild Type mice at 3, 8 and 12 months of age. Learning and memory deficits emerged at 3 months in the 3xTg mouse model, while cellular damage detectable through light microscopy became evident after 12 months. Various parameters were assessed to understand mitochondrial dynamics and morphology alterations. The images identified changes in mitochondrial architecture, such as mitochondrial number, area, and average widths. Additionally, the distance between mitochondria-endoplasmic reticulum contact sites (MERCs) was measured.
Results: Biophysical changes observed in mitochondrial architecture and MERCs shed light on the spatial organization and interactions between mitochondria and the endoplasmic reticulum. This biophysical analysis provides key clues for understanding changes in calcium signaling and cellular communication that affect mitochondrial dynamics and morphology in the CA3 region.
Conclusion: Understanding the complicated link between mitochondrial structure and MERCs in the 3xTg model could aid in the treatment of Alzheimer's disease via therapies aimed at protecting mitochondrial function.

Host: Stefania Raimondo

Monday 4/12/23 - Hybrid Seminar

Fernando De Castro, Cajal Institute, Spanish National Research Council, Madrid, Spain

When Cajal and the Spanish Neurological School showed the World how to study the brain

Santiago Ramón y Cajal (1852-1934) was still young and brimming with vital energy when he was tought in the reazione nera (1887), discovered years before by the Italian histologist Camillo Golgi in 1873. Cajal became absolutely passionated about the fine structure of the nervous system and started one of the last true epics of our modern history: the identification of nervous cells and their organisation to form the brain. His discoveries paving the birth of modern Neuroscience (the individuality of neurons/neuron theory, the synapses and the dendritic spines, the dynamic polarization of the neuron, the growth cones and the chemotactic hypothesis on their navigation…) saw the light while he was full professor at Barcelona (1887-1892): all this work was done by Cajal himself, alone, using his own private money to equip his laboratory… at his own home. Reticularists started to be defeated in 1889, but they were too powerful and stubborn to easily surrender the field to Cajal and the growing number of neuronists. Clairvoyant about the dimension of the scientific feat that he decided to face, as soon as he received international recognition and the Spanish authorities realized, Cajal received a modern laboratory in Madrid and started recruiting a handful of brilliant pupils who also contributed with significant and sometimes decisive discoveries to lay the foundations of modern Neuroscience and Neurology. Among many others, the most distinguished were Tello, Achúcarro, Pío del Río-Hortega, de Castro and Lorente de Nó, who worked side by side with El Maestro: the stud of their discoveries represents one of the zeniths of the collective discoveries ever in the History of Science. Their contributions range from the

discovery of two of the three main types of glial cells to the description of the reverberant circuits that paved the way to Cybernetics, passing through the first identification of arterial chemoreceptors and the classification of nervous tumors. All together, they are known as the Spanish Neurological School, the School of Madrid or, directly, the School of Cajal. In words of Sherrington: “Never has anyone stated out on a great research more single-handed than at his beginning did Cajal. But as the years went by, if ever scientist had a school it was Cajal”.

Host: Alessandro Vercelli

Wednesday 6/12/23 - Hybrid Seminar

John Mitrofanis, Scientific Director, Fonds Clinatec, Université Grenoble Alpes, France
Institute of Ophthalmology, University College London, United Kingdom

Lights-on for neurodegenerative disease: exploring the benefits of photobiomodulation

This seminar takes you on a journey, tracing the history of a somewhat serendipitous finding in the laboratory, to the translation of this finding to the clinic and its use on patients. The journey starts with a discussion over a cup of coffee between two old friends. They devised an experiment using a photobiomodulation device, one that delivered red to near-infrared light, on a few spare parkinsonian mice left over from other experiments. The thinking was that because photobiomodulation stimulates mitochondrial function, it may improve the mitochondrial dysfunction and protect neurones against the neurodegenerative insult (ie parkinsonian). After a week or so, it turned out that these photobiomodulation-treated mice had more surviving neurones than those that were not treated; in addition, they were found to have improved locomotor behaviour. This led to explorations in non-human primates, the gold-standard of all animal models of the disease. Here, in this species, the same beneficial outcomes were found, namely, less pathology and improved clinical signs. These experimental findings led to clinical interest and, as it stands now, clinical trials are underway testing the efficacy of several photobiomoduation approaches in patients. There are also encouraging, early indications that photobiomodulation is effective in Alzheimer's disease, with both neuroprotective and positive cognitive behavioural outcomes being evident in mouse models of the disease. We are in the process of starting a new series of studies that test the efficacy of photobiomodulation in Alzheimer's disease further, in both animal models and in patients.

Host: Corrado Calì