UNIVERSITÀ DI TORINO

FONDAZIONE CAVALIERI OTTOLENGHI

NICO Neuroscience Institute Cavalieri Ottolenghi

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Annual Report 2024

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NICO 2024 by the numbers

10
Research Groups
102 Scientists

40
Peer-Reviewed
Publications

77
Collaborative Initiatives
with
International
Research Groups

59
On-going/Granted
Research Projects

11
Scientific
Conferences/workshops
organized
by NICO members

29
Invited speakers

1
Spin-off Company

1
Biobank

122
Outreach Activities
62 Invited Talks
60 Science Dissemination Initiatives

32
trained
PhD students

4752
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 can be found at the link **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 36 PhD students. Moreover, as lecturers at the schools of Biology, Biotechnology, Pharmacy, Medicine, Psychology and Veterinary Medicine, they are involved in tutoring students in the preparation of theses for bachelor's and master's degrees. Moreover, NICO researchers are directly involved in the organization of the Master's degree in Biotechnology for Neuroscience (of which 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 R. Levi Montalcini, Clinical and Biological Sciences, Veterinary Sciences and Systems Biology. NICO members belonging to the Department 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 "CittadelladelloSpazio" (according the Space Research Platform of UNITO).

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**.

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 2024, Prof. Boido and Dr. Stangaorganised for the third time a biannual international workshop on motoneuron diseases which was attended by 115 people.
NICO researchers are/have been members of committees for national and international meetings and societies and acted as referees for international peer review journals and panels of funding agencies. From January 2022 to December 2023 the NICO scientific director, A. Vercelli was acting as president of the Italian Society for Neuroscience and now serves as past president until end 2025.
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. MariasilviaCiola. In 2022 this led 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.
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, Mnesys, Anthem 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.

On the 10th of November 2024, Fernando Josa Prado and Stefano Zucca organized a workshop on "Mental Health in Academia and Virtuous Academic Culture", under the patronage of ALBA 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 2027). 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**:
AdultNeurogenesis (PIs Luca Bonfanti and Paolo Peretto)
Ageing and Alzheimer's disease (PI Elena Tamagno)
Brain Development and Disease (PI Alessandro Vercelli)
Clinical Neurobiology (interim PI Alessia Di Sapio)
EmbryonicNeurogenesis (PI Ferdinando Di Cunto).
NerveRegeneration (PI Stefania Raimondo – formerly S. Geuna)
Neuroendocrinology (ff PI Stefano Gotti)
Neurophysiology of Neurodegenerative Diseases (PI Filippo Tempia)
Neuropsychopharmacology (ff PI Ilaria Bertocchi)
Physiopathology of Neural 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 SatutiWerdiningsih and MartirDyrmishi).

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. MariasilviaCiola.

According to the Convention with the University of Turin and with San Luigi Hospital of Orbassano, the NICO hosts:
- **University staff**: 6 full professors, 15 associate professors, 9 university research assistants, 3 technicians, 30 post-docs/bursaries and 36 doctoral students;
- **Hospital staff**: 1 manager biologist, 2 specialists in Clinical Biochemistry, 2 post-doc fellows.

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

During 2024, 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

Thanks to the involvement of the Department of Neuroscience R- Levi Montalcini, which was awarded twice the seal of department of excellence, NICO could update its facilities consistently.

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 UltraMicroscopeLaVision/MiltenyiBiotec. 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. The two confocal microscopes will soon be dismissed: one has been substituted by the Zeiss apotome, and the other will soon be substituted by a Zeiss LSM 980 Super-resolution confocal microscope.
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 and transduction. 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 mammalian cells, 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.

For in vivo recordings, systems for multichannel recordings and calcium photometry are in place.

C. elegans facility

The C. elegans laboratory offers all the necessary expertise, instruments and strains necessary to utilize this genetically-tractable animal model for studying disorders involving altered neural development and neurodegeneration. In particular, many strains expressing fluorescent neuro-specific markers, an oocyte-injection setup for production of transgenic strains and three setups for functional assays and behavioral analysis are available.

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.

Personnel
New personnel were recruited by the University Departments collaborating at NICO: the 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. 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.

Upcoming projects on instrumentation, personnel and facilities
To further promote the implementation of instruments, the Scientific Director applied to Regione Piemonte for an implementation of the microscopy facilities, together with Polytechnic of Turin.

Some considerations regarding research funding
Members of the NICO have raised in the year 2024 more than 7.5 million € in grants for UNITO and 0.4 million € for FCO. 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: from October 2024 Prof. Boido is the leader of the Spoke 5 "Industry for Health and Silver Economy". 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 is 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. Also, prof. Boido, Buffo and Vercelli received three cascade grants from PNRR projects (Mnesys and Anthem)and Prof. Di Cunto participates to a PNRR-TR1-2023 project.
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 (250.000 € in 2024). 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 Ministry 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 (under renewal) foresees a yearly contribution of 25.000 € to FCO.

Fund raising
In 2024 the collaboration with fundraising consultant Dr. Alessandra Gerbo went on. The main objectives of her work were to develop further relations with philanthropic foundations and other stakeholders with a potential role in helping the Foundation’s fundraising, to coordinate the NICO 5 x 1000 campaign and to ensure the development of the new website include the fundraising

perspective. Dr. Gerbo also worked to the submission of some project requests in response to call issued by local philanthropic foundations. The most relevant development in this respect was the winning of an award of 50.000 € from Compagnia di San Paolo's Next Generation You Program. The amount will be dedicated to an organisational strengthening programme lasting 18 months (from December 2024 until mid 2026). The programme, written in collaboration with ON! Srl consultants (that will also help its implementation) will focus on many aspects strictly connected with fundraising: strategic positioning and articulation of NICO's value proposition in relation to different stakeholders, boosting of management skills for key staff members, assessment of internal processes and their improvement in order to gain a more strategic vision and a greater efficiency in planning and decision-making.

PLANS FOR INTERNATIONALIZATION

During 2024, the internationalisationprogramme began to take shape and the first achievements were accomplished.

As far as the Neuroscience Institute of excellence Network – NIN is concerned, the first two agreements were signed with the Instituto Cajal in Madrid and IDOR in Rio de Janeiro. Moreover, talks are ongoing with the Neuroscience Department of the University of Osaka (Japan) and the Centre for Cognition and Sociality of the Institute for Basic Science - IBS (Republic of Korea).

Prof. Vercelli served as vice-Rector for Biomedical Research until 2024 and in this capacity, he participated in other institutional missions of University of Torino. In particular, on the occasion of the second mission to South Korea (September 2024), agreements were finalised with IBS both for the start of collaboration at PhD level and for the participation of IBS neuroscience centres in the network promoted by NICO.

New collaborations for NICO researchers were also made possible thanks to the director's participation in the institutional mission to Singapore in June 2024. Webinars with the National University of Singapore Medical School took place in the second half of the year.

The agreement with 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) was signed in March 2024 and both the University and NICO, represented by the director, are working on an initiative to be held in Torino during 2025 to be proposed to partners.

Despite the serious situation in the Middle East, thanks to the initiative of the Italian Embassy in Tel Aviv, contacts with several Israeli researchers were resumed during the second half of 2024 and a couple of them managed to come to Torino for meetings with both the Department of Neuroscience and NICO. Hopefully during 2025, collaboration with Israeli institutions, in particular, the Weizmann Institute, the University of Haifa and Tel Aviv University will continue.

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 2024
• UniStem Day 2024 (22 March);
• Olympic Games of Neuroscience Piedmont (February 7 - March 9);
• Brain Awareness Week (March 12-15) dedicated to Ferdinando Rossi, 10 years after his death + Ferdinando Rossi Lecture on Neuroscience with Prof. Monica De Luca (SINS's President)
• 5xmille Campaign (on web and social media, from April to September)
• Pint of Science (Scientific events in pubs May 13-15)
• European Researcher's Night UNIGHT (September 27-28) 6 Scientific Cafes and more
• Festival dell'Innovazione e della Scienza Settimo T.se (October 6-13) 3 Scientific Cafes and 2 Conferences
• TrendSanità Policy and Procurement in Health Care (on line and in presence Conferences)
• Web (italian research news and news || english research news) and Social media dissemination (Facebook - Instagram - LinkedIn - YouTube)

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 Olympic Games of 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 - Sportello Lesionispinali) 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 GirotondoOnlus 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, international event; NICO organizes each year the Turin edition), Aula Magna of Palazzetto Aldo Moro (with 400 students of the secondary school).

SCIENTIFIC SEMINARS AT NICO
25 seminars were held on Friday afternoons. For invited speakers, see the attached list.

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 perspective, in 2026 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: to this aim, we are developing a project funded by Compagnia di San Paolo in the frame of the program NGY.

Finally, following the requests of the Board of Auditors, and of the Council of Administration, the Foundation is preparing a regulation and a website for transparency in the public administration, to comply anti-corruption rules by the ANAC.

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 2024

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

Email: luca.bonfanti@unito.it

Principal Investigator 2

PAOLO PERETTO

Degree: PhD Birthdate: 18/09/1963

Nationality: Italian Gender: M

Phone: 00 39 011 6706605

Email: paolo.peretto@unito.it

Personnel

1. SILVIA DE MARCHIS

Degree: PhD Birthdate: 14/09/1966

Nationality: Italian Gender: F

Phone: 00 39 011 6706605

Email: silvia.demarchis@unito.it

Position: Associate professor

Role & Expertise: Lead researcher on postnatal neurogenesis in mouse models

2. FEDERICO LUZZATI

Degree: PhD Birthdate: 20/10/1974

Nationality: Italian Gender: M

Phone: 00 39 011 6706615

Email: federico.luzzati@unito.it

Position: Associate 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: Assistant professor (RTD-A)

Role & Expertise: Cellular and molecular analyses of AN in the olfactory bulb and 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: MSCA Post-doctoral fellow

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

6. ALESSANDRA STELLA

Degree: PhD Birthdate: 24/06/1991

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 PRIN)

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)- Assegnista di Ricerca since February 2024

Role & Expertise: Cellular and molecular analyses of lesion-induced neurogenesis & analysis of human brain organoids

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 DALLORTO

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, Western blot analysis, confocal & super-resolution microscopy, morphometric analysis, mouse models, human iPSCs derived neurons in vitro.

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

12. FLAVIA MARIA DOMIZIA DI FIORE

Degree: Chemistry and Pharmaceutical Technology Birthdate: 13/03/1998

Nationality: Italian Gender: F

Phone:00 39 011 6706632

Email: flaviamariadomizia.difiore@unito.it

Position: PhD student (40° cycle)

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

13. IKHLASS BELHASSEN (since August 2024)

Degree: MSc in Genomics and Proteomics Birthdate: 09/03/1996

Nationality: Tunisian Gender: F

Phone:

Email: ikhlass.belhassen@unito.it

Position: Assegnista di Ricerca

Role & Expertise: Biochemical and morphometric analysis on genetic mouse 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
May 2022 – April 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	Trapezio – Compagnia di San Paolo	Coordinator	30,000 Euro	UNITO
October 2022 – July 2025	Neuroni 'immaturi' come riserva di cellule indifferenziate 'dormienti' nella corteccia cerebrale umana	Luca Bonfanti	Fondazione CRT (bandiordinari)	Coordinator	27,000 Euro	FCO
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	PRIN 2022	Coordinator	65,000 Euro	UNITO
November 2023-October 2025	Nr2f1-dependent regulation of Mitochondrial Function in Neural Development	Silvia De Marchis	PRIN 2022	Coordinator	71,466	UNITO

	and Disease					
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 in sexual imprinting	Serena Bovetti	Human Frontier Science Program	Coordinator	350.000 $/3 years	UNITO
March 2022-Nov 2024	Imprinted SCENTs: odour control of mate preference	Serena Bovetti	Trapezio – Compagnia di San Paolo	Coordinator	30000 euro	UNITO
January 2023- dec 2024	Multimodal integration of olfactory and acoustic cues in mouse courtship communication	Serena Bovetti/Stefano Zucca	H2020 Marie Sklodowska Curie Action Individual Fellowship	Coordinator / Recipient	39.000 euro	UNITO
2023-2025	Discovering the Effectors of Lifestyle-driven Memory enhancement via Inflammation	Serena Bovetti	PRIN-PNRR 2022	PI	81.000 euro	UNITO
Sept2024-Jan2027	Malattie del neurosviluppo: studio dei meccanismi patogenetici e individuazione di nuovi potenziali target terapeutici	Sara Bonzano	Fondazione CRT (Bando Erogazioni Ordinarie 2024 – I tornata)	Coordinator	30.000 Euro	UNITO

3. SCIENTIFIC ACTIVITIES IN 2024

Luca Bonfanti, Associate professor (PI)

Supervised PhD students:	Alessia Pattaro (second year) Marco Ghibaudi (Assegno di ricerca PRIN)
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:	- *Brain structural plasticity: from mice to humans* (seminar at the Dept of Psychology, Turin, Hosted by Olga Dal Monte)
● Science communication:	- *Plasticità cerebrale, giovani e futuro* (conference in: Scienza sotto i campanili, Asti) - *Plasticità cerebrale: mi modifico, dunque sono* (conference in: Festival Connessioni - Profondo umano, Alba) - *Dagli studi sul cervello alle ricadute sull'apprendimento (*Intervento di apertura al Meeting annuale dell'ANP (Associazione Nazionale Dirigenti Pubblici e Alte Professionalità della Scuola, Roma, 8-9 ottobre 2024) - *Lezione nel corso di Neurodidattica TRECCANI* (Le Neuroscienze e il cervello che cambia) - *Neuroni immaturi: la plasticità incontra l'evoluzione* (Caffè scientifico a UNIGHT 2024, Giardini Reali, Torino) - *Neuroni immaturi: la plasticità incontra l'evoluzione* (Caffè scientifico al Festival della Scienza e dell'Innovazione, Settimo Torinese) - *Come il cervello "scolpisce" la visione del mondo* (Articolo divulgativo per Rivoluzione Positiva)
● Editorial duties:	- Editor in chief *Frontiers in Neurogenesis* - Reviewing editor in *Frontiers in Mammal Science* - Joined the Editorial Board of *Regenerative Medicine Reports* (Walters Kluwer)
● others	- *Membership in the Cost action LIFT* (Lifting Farm Animal Lives – laying the foundations for positive animal welfare - CA21124) - *Membership in the Cost action AFFECT-EVO* (An Evolutionary View to Understanding Affective States across Species - CA23106) - Reports on career advancements of researchers: *Shawn F. Sorrells*, University of Pittsburgh
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:	Flavia Maria Domizia Di Fiore (co-tutor with S. Bovetti)
Honors, prizes, awards:	n.a.
Outreach activities	
● International collaborations:	Dustin Penn (Konrad Lorenz Institute of Ethology, Veterinary Medicine University, Vienna); Paolo Giacobini (Inserm, UDSL, School of Medicine, Lille, France).
● Invited talks:	
● Science communication:	- I Vertebrati: "Un Giorno all'Università" 2023-2024; 16 Aprile 2024- Bloom Fest del Progetto Living Corridor all'Orto della SME: *come il nostro cervello percepisce i profumi*; 26 Novembre 2024 - " Aldo Fasolo Maestro di Biologia", Accademia Delle Scienze di Torino: *L'evoluzione del laboratorio Fasolo*.
● Editorial duties:	Associate Editor Frontiers in Neuroscience - Referee for Scientific Journals
● others	69° Convegno GEI-SIBSC 2024- session 6, chair "Neurodevelopment"
Organizational activities and responsibilities at NICO:	Representative of the personnel for safety
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	
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:	Scientific Label 2024 from Università Italo-Francese (UIF)
Outreach activities	
● International collaborations:	Michèle Studer, INSERM U636, Nice Sophia Antipolis, France; Wojciech Krezel INSERM, IGBMC, Strasbourg, France. Christian Schaaf, Institute for Human Genetics, Heidelberg, Germany
● Invited talks:	*Exploring Pathogenic Mechanisms of BBSOAS Neurodevelopmental Disorder: Insights from the Adult Mouse Dentate Gyrus Neurogenic Niche* - 69 GEI meeting - Naples 11-14 June 2024.
● Science communication:	- Organizzazione del workshop "*Parole e immagini: percorsi di divulgazione scientifica*" e cerimonia del Premio Aldo Fasolo per la divulgazione in neuroscienze - 7 giugno 2024 - nell'ambito della 27 edizione Cinemambiente -"*Esplorando la Neurogenesi Adulta alla ricerca dei meccanismi associati alle malattie del neurosviluppo*", Accademia delle Scienze di Torino - 26 Novembre 2024 in occasione dell'evento: " Aldo Fasolo Maestro di Biologia".
● Editorial duties:	Referee for Neurobiology of Disease;

● others	-Member of the Scientific Advisory Board of the Jérôme Lejeune Fondation - Referee for grants. -Referee for The University of Cambridge Academic Career Pathways. -Member of the PhD committee of the Ph.D. Course in Biology XXXVII cycle (Giuliana Giamundo)
Organizational activities and responsibilities at NICO:	
Speakers invited:	- Michèle Studer (Nice Sophia Antipolis, France) "In vitro and in vivo modelling of an emerging neurodevelopmental disorder" 31/05/2024 -Elisa Galliano (University of Cambridge, UK) "Neuronal heterogeneity and plasticity in the olfactory bulb" 05/06/2024 -Tudor Badea (Facultatea de Medicina UniversitateaTransilvania din Brașov; Romania) "Genetic analysis of neuronal circuits with cell type resolution: development, function and pathology" 27/09/2024
Other organizational activities:	Coordinator of the teaching committee of the PhD program in Neuroscience.
Workshops, Schools or Conferences organized:	- Organizer and chair of the Symposium "*Mitochondria: Key Players in Neuronal Function and Physiopathology of Neurological Disease*" FENS June 2024, Vienna (AU). -Organizer and chair of the Symposium "*Mechanisms and Therapies of Metabolic and Neurological Disorders*" accepted for the NeuroFrance International Meeting to be held in Montpellier (FR) in May 2025.
Technology transfer achievements (patents, etc.):	

Serena Bovetti, Associate professor, Lead researcher

Supervised PhD students:	Ilaria Ghia (co-tutor with Silvia De Marchis) Flavia Maria Dovizia Di Fiore (co-tutor with Paolo Peretto)
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:	
● Science communication:	-"*Mi piace il tuo profumo*": imprinting e memoria olfattiva nel comportamento riproduttivo". Aldo Fasolo, maestro di Biologia, 26 Novembre 2024.
● Editorial duties:	- Reviewing editor for *Frontiers in Neural Circuits, Molecular Neurobiology, European Journal of Neuroscience*
● others	-External PhD Advisor for: Marta Ribodio; Anita Romiti, Emma Merlin -Member of the PhD committee, dott. LIVIA VIGNOZZI (University of Padua). -Referee for the MSCA Horizon Europe applications
Organizational activities and	Responsible of the two-photon microscope

responsibilities at NICO:	Responsible of the light-sheet microscope Responsible of the BSL2 surgical room
Speakers invited:	- Dr. Cathie Ventalon (ENS, Paris,France) - Prof. Fabio Benfenati (iit, Genova) - Dr. Elena Giglia (UniTO)
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Federico Luzzati, Associate professor, Lead researcher

Supervised PhD students:	
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:	-Webinar: "*Cellule staminali del cervello: Tra sogni di rigenerazione e dura realtà*" organized by Intesa San Paolo Innovation center -"*Una nessuna centomila cellule staminali addormentate nel cervello adulto*", Accademia delle Scienze di Torino - 26 November 2024 for the event: " Aldo Fasolo Maestro di Biologia". -Moderator for the conference of Jordi Manuello "*Segnali dal cervello*" for the series of Giovedì Scienza, organized by Centro Scienza, 28 November 2024
● Editorial duties:	Referee for international journals
● others	
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Sara Bonzano – Assistant professor RTD-A

Supervised PhD students:	Eleonora Dallorto (with Silvia De Marchis)
Honors, prizes, awards:	
Outreach activities	
● International collaborations:	M. Studer (iBV, UCA, CNRS, INSERM - Nice, France) K. Wojciech, Q. Brassart (INSERM, IGBMC - Strasbourg, France) R. Berckervordersandforth, D.C. Lie (FAU - Erlangen, Germany)

● Invited talks:	"*Nr2f1 and Mitochondrial Shaping in the physiopathology of the neurodevelopmental disorder BBSOAS*" Symposium at FENS Forum 2024 - Vienna, 25-28/06/2024
● Science communication:	Short video for the Campagnia 5x1000 - NICO (Instagram)
● Editorial duties:	Referee for: Journal of Experimental Neuroscience Frontiers in Neuroscience (Neurogenesis)
● others	- Bonzano S*, Dallorto E.*, Bertacchi M., Studer M., De Marchis S. FENS Forum 2024 – Sitges (Spain), October 27-29 2024 (selected poster, presented by E. Dallorto) "*Exploring mitochondria function and dynamics in the pathophysiology of the neurodevelopmental disorder BBSOAS*".
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:	
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:	"Workshop: The Social Brain" (Baeza 2024) – "*Whole-brain representation of multimodal courtship cues*".
● Science communication:	- Settimana dello Studente – "*Il corteggiamento con i suoni e odori: come il cervello ci aiuta nella scelta del partner*". - UNIGHT Notte Europea delle Ricercatrici e dei Ricercatori 2024 "*I cinque sensi dell'amore: come il cervello ci guida nella scelta del partner*"
● Editorial duties:	
● others	Poster Presentation: ICN 2024 – International Conference of Neuroethology, Berlin. "*Whole-brain representation of multimodal courtship cues*".
Organizational activities and responsibilities at NICO:	Responsible for the in vivo electrophysiology setup
Speakers invited:	-Dr. Brizio, Università di Torin: "Promoting a culture of clear communication in the lab" -DragonFly Organization: "Intro to Mental Health Literacy" and "Understanding the psychology of Impostor phenomenon" -Prof. Paola Rocca: "Mental health in academia & research culture".
Other organizational activities:	

Workshops, Schools or Conferences organized:	Workshop Organizer: "*Mental Health in Academia and Virtuous Academic Culture*"
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:	
● Science communication:	UNIGHT – Notte Europea delle Ricercatrici e dei Ricercatori 2024 – "*I cinque sensi dell'amore: come il cervello ci guida nella scelta del partner*"
● Editorial duties:	Reviewer for Frontiers of Computational Neuroscience
● others	-Poster at the FENS Conference, Vienna (Austria): *A functional network analysis of the mouse brain: Insights into sexual imprinting.* June 2024
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:	Best Presentation Award at National Meeting of PhD in Neuroscience 2024. November 12th, Naples (Italy).
Outreach activities	
● International collaborations:	
● Invited talks:	I. Ghia, M. Pieroni, S. Zucca, N. Mancin, P. Peretto, S. De Marchis, S. Bovetti. *A multidisciplinary approach to address olfactory dopaminergic population role in processing sexual odors.* National Meeting of PhD in Neuroscience 2024. November 12th, Naples (Italy).
● Science communication:	Talks at science communication nights "Ricercatori alla Spina". U-NIGHT Notte dei ricercatori 2024. Staff for science communication festival "Pint of Science".
● Editorial duties:	
● others	I. Ghia, M. Pieroni, S. Zucca, N. Mancin, A. Pignatelli, M. Barbieri, P. Peretto, S. De Marchis, S. Bovetti. *Investigating the role of the mouse olfactory dopaminergic cells in processing sexual odors*. Poster at FENS Forum 2024. June 25th-29th, Wien (Austria).

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:	"EnricaMarzola" Award for PhD students in Neuroscience
Outreach activities	
● International collaborations:	Michèle Studer (iBV, UCA, CNRS, INSERM - Nice, France)
● Invited talks:	Oral Presentation: Dallorto E., Bonzano S., Bertacchi M., Hidisoglu E., Casile A., Bonifazio G., Marcantoni A., Sassoé-Pognetto M., Studer M., De Marchis S. JEDNs 2024,Doctorale de Nice 21-23 May, 2024 NICE (France) Journées de l'Ecole.; "*Exploring physiopathological mechanisms of the neurodevelopmental disorder BBSOAS: insights from the adult mouse brain and human iPSCs*".
● Science communication:	UNIGHT: la notte dellaricerca 27/09/2024 (member of the organizing committee of the PhD students in Neuroscience)
● Editorial duties:	
● others	Poster presentations: - Dallorto E., Bonzano S., Bertacchi M., Hidisoglu E., Marcantoni A., Sassoé-Pognetto M., Studer M., De Marchis S. FENS Forum 2024 – Vienna (Austria), June 25-29 2024 (selected poster) "*Decreased synaptic GABAergic inhibition in the dentate gyrus of a mouse model of the neurodevelopmental disorder BBSOAS*". - Bonzano S*, Dallorto E.*, Bertacchi M., Studer M., De Marchis S. FENS Forum 2024 – Sitges (Spain), October 27-29 2024 (selected poster) "*Exploring mitochondria function and dynamics in the pathophysiology of the neurodevelopmental disorder BBSOAS*".
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:	Workshop "*How to write a grant*" (Advanced course organized by PhD Student Representatives). June 13th-14th 2024
Technology transfer achievements (patents, etc.):	

Marco Fogli, PhD student / Assegnista di Ricerca

Supervised PhD students:	
Honors, prizes, awards:	
Outreach activities	
● International collaborations:	Philip Greulich (University of Southampton)
● Invited talks:	
● Science communication:	"Just Neuroblast - Disponibilinuovineuroni on demand". Ricercatori alla spina – Brain edition. Casa del quartiere, Turin, 12th March 2024.
● Editorial duties:	na
● others	Oral presentation: *"The widespread neurogenic potential of the adult brain".*NICO NeuroWebinar - Neuroscience Institute Cavalieri Ottolenghi, Orbassano (TO, Italy), 15 November 2024. Poster presentation and participation: - *"Spatio-temporal dynamics of striatal astrocyte neurogenic activation after injury reveal widespread potential beyond known niches".* M. Fogli, G. Nato, P. Greulich, J. Pinto, P. Peretto, A. Buffo and F. Luzzati. "FENS Forum 2024". Vienna (Austria), 25-29 June 2024. And "Cajal's Challenge accepted", Pre-FENS workshop, Vienna (Austria), 24 June 2024. - *"Astrocyte-generated neurons functionally integrate into the lesioned striatum".* G. Nato, M. Fogli, N. Marichal, I. Ghia, G. Zanotto, P. Peretto, B. Berninger, A. Buffo, F. Luzzati. "FENS Forum 2024". Vienna (Austria), 25-29 June 2024. And "Cajal's Challenge accepted", Pre-FENS workshop, Vienna (Austria), 24 June 2024. - *"Lesion induced neuroblasts in the striatum are LGE-class interneurons and are not fated towards adult striatal neuron cell types"*. G. Nato, M. Fogli, V. Cerrato, N. Marichal, V. Proserpio, I. Ghia, S. Oliviero, P. Peretto, B. Berninger, A. Buffo, F. Luzzati. "FENS Forum 2024". Vienna (Austria), 25-29 June 2024. And "Cajal's Challenge accepted", Pre-FENS workshop, Vienna (Austria), 24 June 2024. Membership of Scientific Societies: Federation of European Neuroscience Societies (FENS)
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:	Alessia Pattaro (with Luca Bonfanti)
Honors, prizes, awards:	
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:	*"Immature" neurons in mammals: phylogenetic variation in brian regions and ages* - Seminar at Pittsburgh University (Shawn Sorrells Lab; Pittsburgh, PA) - 06/09/2024
● Science communication:	na
● Editorial duties:	na
● others	Posters: -Ghibaudi M., Telitysin N., Graic JM., Amrein I., Sherwood CC., Bonfanti L. *Non-dividing "immature" neurons in subcortical brain regions of mammals display phylogenetic variation with clear prevalence in primates*. "FENS Forum 2024". Vienna (Austria), 25-29 June 2024 -Pattaro A., Ghibaudi M., Sherwood C. C., Bonfanti L. "*DCX-positive layer II cortical immature neurons in mammals covaries with brain size: new data from dog, horse, and macaque*". Greater Baltimore Society for Neuroscience (GBSfN). 8th November 2024, Baltimore (USA)
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:	na
● Science communication:	na
● Editorial duties:	na
● others	Poster presentation: Pattaro A., Ghibaudi M., Sherwood C. C., Bonfanti L. "*DCX-positive layer II cortical immature neurons in mammals covarieswith brain size: new data from dog, horse, and macaque*". Greater Baltimore Society for Neuroscience (GBSfN). 8th November 2024, Baltimore (USA)
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Other organizational activities:	na
Workshops, Schools or Conferences organized:	na
Technology transfer achievements	na

(patents, etc.):	

Flavia M. D. Di Fiore, PhD student

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

4. Research activity in 2024

a. Summary

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 mechanisms underlying neuronal changes in the hippocampal neurogenic niche across different pathological mouse models
iv) the mechanisms and dynamics of lesion-induced acquisition of a neurogenic competence in striatal astrocytes.

b. Background and rationale

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 neurogenic potential of the mature brain 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 2024 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. These memories involve different type of sensory cues, including olfactory and auditory signals whose processing is integrated into brain circuits. During this year, we analysed the brain areas activated by exposure to single (olfactory or auditory) and multisensory (olfactory and auditory) signals and correlated the results with behavioural performance. We also analysed at what age during development mice start to process USV sounds (Zucca et al., 2024). Finally, we completed our collaboration with Dr. Giacobini (INSERM, Lille) on the role of olfactory GnRH neurons in mating behaviour (Decoster et al., 2024).
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. The neurogenic niche of the hippocampal dentate gyrus (DG) is one main site of postnatal neuroplasticity in which neurogenesis persists throughout life. Adult-born neurons play different physiological roles in learning, memory, pattern separation, and cognitive flexibility, which are altered in neuropathological conditions. Understanding the mechanisms underlying the alterations in adult neurogenesis in animal models of neurological disorders is key in identifying the cellular/molecular targets, a prerequisite neededto develop new interventions aimed at improving cognitive functions.

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 analyzed 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

i) Examine the brain regions involved in the formation of sexual imprinting with specific focus on multimodal olfactory and acoustic integration.
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 animal models of the neurodevelopmental disorder BBSOAS and of neuroinflammation.
iv) Determine the dynamics and mechanisms of striatal astrocytes neurogenic potential and analyze the identity and integration capacity of their neuronal progeny.

d. Results

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 either odors,

USVs or a combination of both, isolated 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. Specifically, we identified the Infralimbic area as a possible hub for multisensory integration and we are now using in vivo miniscope recording on freely moving mice to assess cell activity during exposure to different olfactory and auditory stimuli.

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) Adult DG neurogenesis. We explored the link between mitochondrial dysfunction, adult hippocampal neurogenesis and genes associated with cognitive deficits in neurodevelopmental disorders. We provided insights into how alterations in the transcriptional regulator NR2F1 affect mitochondrial dynamics and may contribute to the pathophysiology of the emerging neurodevelopmental disorder Bosch-Boonstra-Schaaf optic atrophy syndrome (BBSOAS OMIM# 615722; ORPHA 401777). We have also investigated the impact of tamoxifen on the adult mouse dentate gyrus neurogenic niche both in "normal" conditions and upon neuroinflammation. Tamoxifen-inducible Cre-LoxP genetically modified mice are key tools to selectively delete specific genes in a time and lineage restricted manner. However, tamoxifen also exerts off-target effects, whose consequences in the adult neurogenic niche are still poorly addressed. We demonstrated that a two-day tamoxifen does not alter adult neurogenesis in control conditions, but it is effective in counteracting the increase in microglia, astrocytes and radial glial cells with concomitant reduction of newborn neurons observed following LPS-induced neuroinflammation. Through selective microglia depletion, we elucidated that both LPS and tamoxifen influenced astrocytes and radial glial cells via microglia mediated mechanisms, while the effects on neurogenesis persisted even in a microglia-depleted environment.

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 transiently exists during development.

e. Advancement in the field

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) *Adult DG neurogenesis.*We have demonstrated a new role for NR2F1 in the mitochondrial gene expression regulatory network in neurons and support the involvement of mitochondrial dysfunction in BBSOAS pathogenesis. We demonstrated that tamoxifen treatment per se does not alter adult neurogenesis but does modulate cellular responses to inflammatory stimuli exerting a protective role within the adult hippocampal neurogenic niche.

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 generate a specific transient cell type that integrate in pre-existing circuits and may play a role in compensatory plasticity after lesion.

f. Publications

1. Decoster L, Trova S, Zucca S, Bulk J, Gouveia A, Ternier G, Lhomme T, Legrand A, Gallet S, Boehm U, Wyatt A, Wahl V, Wartenberg P, Hrabovszky E, Rácz G, Luzzati F, Nato G, Fogli M, Peretto P, Schriever SC, Bernecker M, Pfluger PT, Steculorum SM, Bovetti S, Rasika S, Prevot V, Silva MSB, Giacobini P (2024) A GnRH neuronal population in the olfactory bulb translates socially relevant odors into reproductive behavior in male mice. Nat Neurosci. 2024 Sep;27(9):1758-1773. doi: 10.1038/s41593-024-01724-1.
Research article – Q1

2. Crisci I, Bonzano S, Nicolas Z, Dallorto E, Peretto P, Krezel W, De Marchis S (2024) Tamoxifen exerts direct and microglia-mediated effects preventing neuroinflammatory changes in the adult mouse hippocampal neurogenic niche. Glia. 2024 Jul;72(7):1273-1289. doi: 10.1002/glia.24526.
Research article – Q1

3. Zucca S, La Rosa C, Fellin T, Peretto P, Bovetti S Developmental encoding of natural sounds in the mouse auditory cortex (2024) Cereb Cortex. 2024 Nov 5;34(11):bhae438. doi: 10.1093/cercor/bhae438.
Research article – Q1

4. Fogli M, Nato G, Greulich P, Pinto J, Ribodino M, Valsania G, Peretto P, Buffo A, Luzzati F (2024) Dynamic spatiotemporal activation of a pervasive neurogenic competence in striatal astrocytes supports continuous neurogenesis following injury. Stem Cell Reports. 2024 Oct 8;19(10):1432-1450. doi: 10.1016/j.stemcr.2024.08.006.
Research article – Q1

5. Bonzano S, Dallorto E, Bovetti S, Studer M, De Marchis S (2024) Mitochondrial regulation of adult hippocampal neurogenesis: Insights into neurological function and neurodevelopmental disorders. Neurobiol Dis. 2024 Sep;199:106604. doi: 10.1016/j.nbd.2024.106604.
Review article – Q1

6. Ghibaudi M, Boda E, Bonfanti L (2024) From mice to humans: a need for comparable results in mammalian neuroplasticity. Neural Regen Res 20(2):464-466.
Perspective article – Q1

7. Bovetti S, Bonzano S, Luzzati F, Dati C, De Marchis S, Peretto P. Linking Adult Olfactory Neurogenesis to Social Reproductive Stimuli: Mechanisms and Functions. Int J Mol Sci. 2024 Dec 28;26(1):163. doi: 10.3390/ijms26010163. PMID: 39796023; PMCID: PMC11720170.
Review article – Q1

5. Future directions and objectives for next years

a. Summary

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 neuroprotective effect of tamoxifen in vivo, 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

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 internalcues (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 foundamental cognitive functions (e.g., memory and learning), it is also showing that this mechanism can be significantly heterogenous among

mammals, and probably 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

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

- *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 aninterdisciplinary 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, and human iPSCs-derived neurons in vitro, 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 highly stereotyped spatial and temporal pattern of QA induced neurogenic response 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 verify the role of neuronal activity in the activation of striatal astrocytes neurogenic potential and in the differentiation and survival of the newly generated striatal neurons.

e. Unique features of the project research

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

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 2024

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.

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

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.

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.

Fernando Josa Prado, MSCA Postdoctoral Fellow, PhD, expertise in cellular and molecular biology, 17-11-1985, fernando.josaprado@unito.it. SUMOMyePath - Unveiling the role of SUMOylation as in myelin biology; implication in neurodevelopment and demyelinating pathophysiology

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 KhastkhodaeiArdakani, 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.

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.

Niccoló Di Cintio, Research Fellow, MSc in Neurobiology, 16-11-1992, +393455998634, niccolo.dicintio@unito.it. Dysmyelination and cognitive impairment

Olga Teresa Bianciotto, PhD candidate, MSc in Biotechnology for Neuroscience, 12/06/1999, +393662845620, olgateresa.bianciotto@unito.it. Generation of optimized cerebellar human organoids

Arianna Contato, Research Fellow, MSc in Cellular and Molecular Biology, 24-11-2000, +393496819252, arianna.contato@edu.unito.it. Generation of integrated cellular models to study demyelination and remyelination.

Giada Musso, Research Fellow, MSc in Cellular and Molecular Biology, 27-03-1999, +390116706632, gi.musso@unito.it, pain research and injury signals, embryonic and adult DRG culture, neurite outgrowth assay, molecular biology, imaging. Co-supervised with dr L Marvaldi.

2. CURRENT GRANTS

Starting-end date	Project Title and ID	Beneficiary	Funding Program/Agency	Role of the unit	Overall AmountFunded	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
2023-2024	In vitro and in vivo molecular mapping of cell therapy for Huntington Disease at single-cell resolution	Annalisa Buffo	PRIN2022, MUR	PI of researchunit	98.405 €	UNITO
2024-2026	INITIATE - CreazIone di una BaNca di TessutIfetAli per lo studio del Tessuto nErvoso - INITIATE	Annalisa Buffo	CRT Foundation	PI	50.000 €	UNITO
2024-2025	UNFOLD -A fully humanised integrated cellular system for scalable modeling of inflammatory demyelination	Annalisa Buffo	PNRR MUR – MNESYS_PE00000006_1.	PI	200.000 €	FCO
2024-2030	CUSTOM-MADE NEURONS FOR CELL THERAPY IN PARKINSON'S AND HUNTINGTON'S DISEASE	Annalisa Buffo	ERC Synergy	PI	2.500.000€	UNITO
2023-2025	Targeting oligodendrocyte dysfunctions to treat cognitive	Enrica Boda	Telethon Foundation Multiround 21-24 – Round 1 2022 Track Basic	PI	129,500 €	Telethon

	defects and epilepsy in primary autosomal recessive microcephaly-17 (MCPH17) models (ID: GMR22T1066)					
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 autosomal recessive microcephaly-17 (MCPH17) models (ID: 20224YJBBP)	Enrica Boda	PRIN2022, MUR	PI	90,000	UNITO
2024-2026	SUMOMyePath - Unveiling the role of SUMOylation as novel molecular pathway in myelin biology; implication in neurodevelopment and demyelinating pathophysiology	Fernando Josa Prado	MSCA-PostdoctoralFellowship Grant	Grantee – PI	188,590	UNITO

3. SCIENTIFIC ACTIVITIES IN 2024

Annalisa Buffo, PI

Supervised PhD students:	Maryam Khastkhodaei (co-supervised with E. Boda) Marta Ribodino, Giacomo Turrini (co-supervised with

	Valentina Cerrato), Olga Bianciotto
Honors, prizes, awards:	--
Outreach activities	
• International collaborations:	NSC-Reconstruct network (main collaborators: E Cattaneo, University of Milano, M Parmar, University of Lund; M Goetz, LMU, Muenchen; G Quadrato, University of Southern California; L Telley, University of Lausanne) See also specific collaborations of lab members below.
• Invited talks:	
• Science communication:	UNISTEM Day Torino 2024 – organizer https://www.nico.ottolenghi.unito.it/Scuole/UniStem-Day-Giornata-Staminali/Unistem-Day-2024-Altri-cervelli BRAIN AWARENESS WEEK (La Settimana del Cervello) – organizer https://www.nico.ottolenghi.unito.it/Neuroscienze-per-voi/Settimana-del-Cervello/La-Settimana-del-Cervello-dedicata-a-Ferdinando-Rossi Festival dell'Innovazione e della Scienza 2024 – speaker – 12 ottobre 2024 Il cervello che legge Discussant at "From Procrustes to Mentor: a mythological walk towards Virtuous Academic Culture"- Workshop on Mental Health in Academia & Virtuous Academic Culture. 17-10-2024. NICO-UNITO, Turin.
• Editorial duties:	Member of the Glia Editorial Board Ad hoc reviewer for the following journals in 2024: Glia, Journal of Clinical Investigations, Nature Neuroscience, Science Advances, Journal of Neuroinflammation
• others	Invited Chair at the EMBO Workshop Unlocking human brain complexity using 3D culture and single-cell omics 13 – 16 October 2024 | Capri, Italy Attended meetings: FENS meeting, 25—29 Jun 2024, Wien. Evaluation panel member for the ERA4Health Joint Transnational Call NANOTECMEC 2024 Evaluation panel member for the call: Single cell approaches for personalized medicine, Max Delbrück Center for Molecular Medicine in the Helmholtz Association – Focus area together with the Berlin Institute of Health (BIH) 2024, DE Evaluator for the Medical Research Council: Clinical Research Training Fellowship, UK Evaluator for the Deutscher AkademischerAustauschdienst fellowships, DE Evaluating member for tenure-eligible lecturers in Physiology, University of Barcelona, ES Memberships in Scientific Societies in 2024:

	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) Italian Glia Network ALBA network Co-supervisor of the PhD candidate Garsia Chiara, Course in Molecular Medicine, Curriculum in Gene and Cell Therapy, Vita-Salute San Raffaele University in Milan, Italy. Advisor of the PhD candidate Mattia Battistelli, School in Molecular and Cellular Biology, University of Milan, Italy. External Examiner of the PhD Candidate Giulia Demenego, PhD Programme in Molecular and Experimental Medicine, Humanitas University, Milan, Italy. External Examiner of the PhD Candidate Patricia García Jareño, PhD Programme in Biology, University of Cardiff, UK.
Organizational activities and responsibilities at NICO:	- Deputy Director of NICO - CEO of S&P Brain - Responsible of BLS2 labs at NICO
Speakers invited:	--
Other organizational activities:	
Workshops, Schools or Conferences organized:	--
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:	*Oligodendroglia heterogeneity in response to DNA damage: impact on neurodevelopment and adult brain functions.*5th Symposium on Physiology and Pathology of Neuroglia, Institute of Neurobiology, Universidad National Autonoma de Mexico, Querétaro (Mexico). 10 Oct 2024. *Oligodendroglia heterogeneity in response to DNA damage: impact on neurodevelopment and adult brain functions.* 25th Biennial Meeting of the International Society Developmental Neuroscience (ISDN), Montpellier (France), 24 Sept 2024. *Oligodendroglia heterogeneity in response to DNA damage: impact on neurodevelopment and adult CNS functions.*Sino-German Workshop "Advanced Concepts of Neuron-Glia Interactions",Center for Integrative Physiology and Molecular Medicine (CIPMM) University of Saarland,Hömburg

	(Germany), 3 July 2024.
• Science communication:	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 2024). Sept 30th 2023. Casa del Quartiere, San Salvario, Torino Penne Amiche della Scienza (corrispondenza ed incontri con la 2C della scuola media di Attigliano- Terni): https://sites.google.com/view/penne-amiche-della-scienza Reviewer for the GiovedìScienza Award
• Editorial duties:	Ad-hoc Reviewer for Nat Commun, Science Advances, Front Cell Neurosci
• others	Grant Reviewer for Italian Foundation Multiple Sclerosis (FISM) PhD Thesis reviewer: Dr. Elisa Degl'Innocenti - PhD in Clinical and Translational Sciences, University of Pisa, Italy Dr. Juliana Castro E Silva - PhD in Pharmacological Biomolecular Sciences, University of Milan, Italy PhD Thesis Committee Member: Dr. Giuseppina De Rocco – Dr. Valeria Casiraghi – Dr. Zulkifal Malik. PhD Program in Experimental Medicine and Medical Biotechnologies, XXXVI cycle, University of Milan, Italy. 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): italianglianetwork.wixsite.com/italian-glia-network/ Vice-President of the BraYn (*Brainstorming Research Assembly for Young Neuroscientists)* Association (www.braynassociation.com/) Attended meetings: 6th European Conference on Brain Stimulation in Mental Health, 11-12 April 2024, Lisbon (Portugal). FENS meeting, 25—29 Jun 2024, Wien (Austria). Sino-German Workshop "Advanced Concepts of Neuron-Glia Interactions", University of Saarland, 2-4 July 2024, Hömburg

	(Germany). 25th Meeting of the International Society Developmental Neuroscience (ISDN), 21-24 Sept 2024, Montpellier (France). BraYn 2024, 8-11 Oct 2024, Verona (Italy).
Organizational activities and responsibilities at NICO:	--
Speakers invited:	Prof. Angelisa Frasca – BIOMETRA, University of Milan Prof. Deborah Chiabrando – MBC, University of Turin
Other organizational activities:	Organizer of the annual Plogging walk in the frame of M'Illumino di Meno Day
Workshops, Schools or Conferences organized:	Member of the Steering Committee of the BraYn conference (www.braynconference.com/) First Italian Glia Network (IGN) Symposium (online), 18 Nov 2024.
Technology transfer achievements (patents, etc.):	--

Giulia Nato, Post-doctoral Fellow

Supervised PhD students:	--
Honors, prizes, awards:	--
Outreach activities	
• International collaborations:	Benedikt Berninger (King's College; London), Magdalena Goetz (LMU, Muenchen)
• Invited talks:	--
• Science communication:	For the Brain Awareness week: il *Tram dellascienza*. C'era una volta un neurone…Storie di un groviglio chiamato cervello. In collaboration with Associazione Centroscienza onlus. 15/03/2024
• Editorial duties:	
• others	Attended meetings: FENS forum 2024 25-29 June, Vienna Posters presented: *Astrocyte-generated neurons functionally integrate into the lesioned striatum, - Lesion induced neuroblasts in the striatum are LGE-class interneurons and are not fated towards adult striatal neuron cell types; Spatio-temporal dynamics of striatal astrocyte neurogenic activation after injury reveal widespread potential beyond known niches* FENS travel grant for attending the FENS forum 2024 Membership in Scientific Societies in 2023: Italian Society of Neuroscience (SINS)
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:	Olga Bianciotto (co-supervised with Annalisa buffo)
Honors, prizes, awards:	--
Outreach activities	
• International collaborations:	Fernando De Castro (Cajal Institute, Madrid, Spain) NSC-Reconstruct network (E Cattaneo, University of Milano); Sabah Mozafari (University of Paris, France); Juan GarcìaLeòn (University of Malaga, Spain)
• Invited talks:	
• Science communication:	Local organizer for Pint of Science - Beautiful Mind, May 13th-15th 2024
• Editorial duties:	--
• others	Attended meetings: ISSCR 2024, Hamburg, July 10th– 13th -Poster presented *Modeling Autosomal Dominant Leukodystrophy with human iPS derived glial cells: altered phenotypes and rescue strategies* Membership in Scientific Societies in 2024: Italian Glia Network Italian Society of Neuroscience (SINS) Federation of European Societies (FENS) International Society for Stem Cell Research (ISSCR) ALBA Network
Organizational activities and responsibilities at NICO:	Co-Responsible of the Dissection Room
Speakers invited:	Vasco Meneghini San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), IRCCS San Raffaele Scientific Institute, Milan, Italy – Friday, February 16th, 2024
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:	--
Outreach activities	
• International collaborations:	Prof. Ludovic Telley (University of Lausanne, Switzerland); Prof. Magdalena Götz (LMU, Muenchen, Germany)
• Invited talks:	• A single cell atlas to unveil the diversity of mouse cerebellar astrocytes: insights into their molecular identities, development, and functions", 27th Meeting of The French Glial Cell Club, Sète, October 8-11, 2024. • "A single cell atlas to unveil the diversity of mouse cerebellar astrocytes: insights into their molecular identities, development, and functions", Meeting on Cerebellar development and disease at single cell resolution, Heidelberg Academy of Science, September 11-13, 2024.

• Science communication:	Local organizing committee_ Pint of Science - Beautiful Mind - Turin, May 12nd-13th 2024
• Editorial duties:	Review editor for Frontiers in Neuroscience (section Neurogenesis) Ad hoc reviewer for the following journals in 2024: Frontiers in Cellular Neuroscience, Scientific Reports, Neuroscience, Zoological Research
• others	Attended meetings: • 27th Meeting of The French Glial Cell Club, Sète (France), October 8-11, 2024. • Meeting on Cerebellar development and disease at single cell resolution, Heidelberg Academy of Science, Heidelberg (Germany) September 11-13, 2024. • Stem Cells In Neuroscience meeting, Tübingen (Germany), 11-13 March 2024. Co-Founder and member of the Italian Glia Network (IGN) Membership in Scientific Societies in 2024: Federation of the European Neuroscience Societies (FENS) Italian Society of Neuroscience (SINS) Società Italiana di Fisiologia (SIF) France Cerebellum Club
Organizational activities and responsibilities at NICO:	• Group administrator for access to the Internet and to computing resources at NICO • Responsible of the ZEISS Axio Scan.Z1 use and its workstation at NICO • Member of the Support and Well-being Committee at NICO
Speakers invited:	--
Other organizational activities:	Co-organizer of Pint of Science - Beautiful Mind Team - Turin, May 12nd-13th 2024
Workshops, Schools or Conferences organized:	--
Technology transfer achievements (patents, etc.):	--

Fernando Josa Prado, MSCA Postdoctoral Fellow

Supervised PhD students:	--
Honors, prizes, awards:	--
Outreach activities	
• International collaborations:	Prof. Dr. Arne Battefeld (Univeristy of Bordeaux, France) Dr. Fernando de Castro Soubriet (Cajal Institute, Madrid, Spain)
• Invited talks:	na
• Science communication:	"From Procrustes to Mentor: a mythological walk towards Virtuous Academic Culture"- Workshop on Mental Health in Academia & Virtuous Academic Culture. 17-10-2024. NICO-UNITO, Turin.
• Editorial duties:	*Ad hoc* reviewer for: Plos One; Journal of Cellular and Molecular Medicine
• others	European Commission – REA: Expert - Project evaluator. Special expertise in Researchers Career Development- activated

	for SWAFS and COFUND calls. • EURAXESS projects advisor and collaborator (Currently Science4Refugees; Past – PIPERS, EURAXIND, TOPIV and TOPV) • President of Scientist Returned to Spain Association (CRE; until April 2024) • Member of the Direction Board member of GADEA Science Foundation (Spain) • Member of Spanish and Italian Chapters of Marie Curie Alumni Association • Internationalization advisor for the Madrid's Region Government through Madrid Foundation (Spain). • Board Member (and Founder) of the Association for High-Level Management of Research, Innovation and Tech-transfer Institutions (PADIIT, Spain) • Member of the Internation Committee of the Spanish Planetology and Astrobiology Network (REDESPA, Spain)
Organizational activities and responsibilities at NICO:	--
Speakers invited:	--
Other organizational activities:	Collaborator with the Doctoral School of Unito and EuraxessUniTo (Lucia Salto)
Workshops, Schools or Conferences organized:	Workshop on Mental Health in Academia & Virtuous Academic Culture. 17&18-10-2024. NICO-UNITO, Turin.
Technology transfer achievements (patents, etc.):	--

Marta Ribodino, PhD candidate

Supervised PhD students:	na
Honors, prizes, awards:	--
Outreach activities	
• International collaborations:	Proff. Malin Parmar and prof. Tomas Bjorklund (Lund University, Sweden); Prof. Elena Cattaneo (University of Milano)
• Invited talks:	--
• Science communication:	Neurosciencebooth "Exploring the Human Mind" at Notte Europea delle Ricercatrici e dei Ricercatori, Unight 2024, September 28th, 2024
• Editorial duties:	
• others	Attended meetings: • ISSCR 2024 Conference, July 10th-13th, Hamburgh, Germany – *poster presented: Toward the optimization of human medium spiny neuron grafts in the QA-lesion HD rat model – poster presentation.* • Annual meeting of the NSC-Reconstruct Consortium, April 2024, Bellagio, Italy – *talk: Integration of sg-hMSN grafts in a model of Huntington's Diseaseand development of tools for selective targeting of MSNs* Membership in Scientific Societies in 2024: International Society for Stem Cell Research (ISSCR) Italian Society of Neuroscience (SINS)

	Italian Glia network ALBA Network
Organizational activities and responsibilities at NICO:	--
Speakers invited:	--
Other organizational activities:	--
Workshops, Schools or Conferences organized:	--
Technology transfer achievements (patents, etc.):	--

Martino Bonato, PhD candidate

Supervised PhD students:	na
Honors, prizes, awards:	--
Outreach activities	
• International collaborations:	--
• Invited talks:	BraYn Conference "*Unravelling the roles of oligodendrocyte progenitor cells in the development of the cortical Inhibitory system"* 2024 October 11th 2024 (Verona, Italy) – Speed Talk
• Science communication:	• Ricercatorialla spina - Brain edition, January 24th, March 12th and 27th 2024 • The Green Brain (U*Night - La Notte Europea dei Ricercatori e delle Ricercatrici 2024), September 28th 2024
• Editorial duties:	
• others	Attended meetings: • 27esimo Congresso "Patologia Immune e Malattie Orfane", February 1st-3rd 2024 • FENS forum 2024, June 25-29th 2024 (Vienna, Austria). Presented poster: *Selective behavioral alterations after acute Particulate Matter exposure in a pre-symptomatic Multiple Sclerosis mouse model* • Sino-German Workshop "Advanced Concepts of Neuron-Glia Interactions" - Center for Integrative Physiology and Molecular Medicine (CIPMM) University of Saarland, July 2—3rd 2024 (Hömburg, Germany) Poster presented: *Unravelling the roles of oligodendrocyte progenitor cells in the development of the cortical Inhibitory system* • BraYn Conference 2024 October 9-11th 2024 (Verona, Italy) • First Italian Glia Symposium (18/11/24 Online, Italian Glia Network). Attended webinars and workshops: • "Creatine transporter Deficiency: The long Journey to successful therapy"; Dr. Laura Baroncelli, 24/01/24, Neuroscience PhD program seminar • "Armati di Scienza"; Dr. Elena Cattaneo and Dr. Gabriele Beccaria, 26/01/24, Lezione inaugurale del Master in Comunicazione della Scienza • "Metagenomics", CSQB PhD seminar, September 27th 2024 • "Preserving the Brain: A Call to Action" (16/10/24, Fondazione Praga – Milano, Partecipazione Online)

	Attended Courses: • "Small RNA functions and applications"; CSQB PhD Program activity, June 21st 2024 • Neuroimaging Genetics; Pr. Fabrizio Pizzagalli, CSQB PhD Course, September 12-13th 2024 • Epigenetic Workshop; Pr. Francesco Neri, CSQB PhD Didactic Workshop, October 1st 2024 Membership in Scientific Societies in 2024: Italian Society of Neuroscience (SINS) Italian Glia network 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.):	--

Maryam Khastkhodaei Ardakani, PhD candidate

Supervised PhD students:	na
Honors, prizes, awards:	--
Outreach activities	
• International collaborations:	--
• Invited talks:	--
• Science communication:	Member of the organizing committee for Neuroscience booth "Exploring the Human Mind" at Notte EuropeadelleRicercatrici e deiRicercatori, Unight 2024, September 28th, 2024
• Editorial duties:	
• others	Attended meetings: • Neuroscience 2024, Society for Neuroscience (SfN), October 5th-9th, 2024, Chicago, United States – Poster presented: *Unravelling the roles of oligodendrocyte progenitor cells in the development of the cortical Inhibitory system* • First Italian Glia network (IGN) meeting (Participant) Attendedwebinars: • "NICO NeuroWebinar". Membership in Scientific Societies in 2024: • Society for Neuroscience (SfN) • Federation of the European Neuroscience Societies • (FENS) • Italian Society of Neuroscience (SINS) • Brainstorming Research Assembly for Young Neuroscientists (BraYn) association • ALBA Network Discussant/Peer advisor of PhD student Dr. Emma Merlin-

	Data report of PhD Students (38th cycle)-15th February 2024, Turin, Italy.
Organizational activities and responsibilities at NICO:	--
Speakers invited:	--
Other organizational activities:	--
Workshops, Schools or Conferences organized:	--
Technology transfer achievements (patents, etc.):	--

Giacomo Turrini, PhD candidate

Supervised PhD students:	na
Honors, prizes, awards:	--
Outreach activities	
• International collaborations:	Ludovic Telley (University of Lausanne)
• Invited talks:	First Italian Glia Network (IGN) meeting, 18th November 2024. Selected talk: *Transcriptional heterogeneity of cerebellar astrocytes unveiled by single cell and spatial transcriptomics*
• Science communication:	• Ricercatori alla Spina – Brain edition, 12th March 2024, Casa del Quartiere, Turin • Member of the local committee for Pint of Science, 15th May 2024, Birrificio Torino, Turin. • Member of the organizing committee for Neuroscience booth "Exploring the Human Mind" at Notte EuropeadelleRicercatrici e deiRicercatori, Unight 2024, September 28th, 2024
• Editorial duties:	--
• others	Attended meetings: ● Cerebellar Development and Disease at Single Cell Resolution, Heidelberg, 11th,12th,13th September 2024. Selected poster: *Molecular diversity of adult mouse cerebellar astrocytes* ● First Italian Glia Symposium, first Italian Glia Network (IGN) meeting, 18th November 2024. Selected talk: *Transcriptional heterogeneity of cerebellar astrocytes unveiled by single cell and spatial transcriptomics* Attended educational courses: ● Broad Neuroscience training: Clinical, methodological and theoretical aspects of translational and cognitive neuroscience - Toolbox Course, 10th January 2024. ● PhD Seminar Laura Baroncelli: "Creatine Transporter Deficiency: the long journey to successful therapy", 24th January 2024 ● PhD Seminar Silvia Cappello: Cellular Crosstalk in Neurodevelopmental Disorders, 21st February 2024 ● From neural cell excitability to brain circuit recording, visualization and manipulation (advanced course), 14th,23rd,28th February, 6th,13th, 20th March 2024. ● Introduction to Research Methodology (toolbox course) 8th,15th, 22th March 2024 ● Ethics of research and Responsible research: an introduction

	(toolbox course) 20th, 21st May 2024 Attended workshops: ● Genomica e TecnologieAvanzate – FISV Course, 25th,26th January 2024 ● Selected participant at Mathematics of Life - EMBL Course, 11th-15th March 2024 ● Selected participant at Lipari School on Computational Life Sciences - Jacob T. Schwartz International School for Scientific Research, 22nd-26th July 2024 Memberships: ● Italian Glia Network
Organizational activities and responsibilities at NICO:	--
Speakers invited:	--
Other organizational activities:	--
Workshops, Schools or Conferences organized:	--
Technology transfer achievements (patents, etc.):	--

Niccoló Di Cintio, Research Fellow

Supervised PhD students:	na
Honors, prizes, awards:	--
Outreach activities	
• International collaborations:	--
• Invited talks:	--
• Science communication:	--
• Editorial duties:	--
• others	Attended courses: • Combining Fluorescence Microscopy and 3D Reconstruction to Investigate Neuron-Glia Interaction in Neurological Diseases, 10 December 2024, 5, online course, held by Oxford Instruments • National Legislation and Ethics 9 and 12 December 2024 – held by I.T.S. Teramo, online course • FISV Course "Genomics: Advanced Technologies" for Young Researchers, 25-26 January 2024, 9:00 a.m. to 6:00 p.m., Milan, held by FederazioneItalianaScienzedella Vita. Seminars and Webinars: • Dr. Laura Baroncelli, “Creatine Transporter Deficiency: The Long Journey to Successful Therapy”, 24 January 2024, Anatomy Institute, Aula Magna, Corso Massimo d'Azeglio 52, Torino • Prof. Silvia Cappello, “Cellular Crosstalk in Neurodevelopmental Disorders”21 February 2024, Anatomy Institute, Aula Magna, Corso Massimo d'Azeglio 52, Torino • Prof. Rossella Di Giaimo, “Deciphering Pathological Mechanisms in a Neurodevelopmental Disease: Insights from Extracellular Vesicles”, 26 September 2024, p.m., Molecular

	Biotechnology Center, Via Nizza 52, Torino • NICO NeuroWebinar - periodically at 2.00 p.m.- https://www.nico.ottolenghi.unito.it/Agenda/NICO-NeuroWebinar Attended Meetings: • FENS Forum, 25- 29 June 2024, Vienna (Austria), poster presented: *Female brain and healthy aging: the pivotal role of NPY-1R regulation; Selective behavioral alterations after acute Particulate Matter exposure in a presymptomatic Multiple Sclerosis mouse model; Unravelling the roles of oligodendrocyte progenitor cells in the development of the cortical Inhibitory system.* • BraYn 2024- 7th Edition brainstorming research assembly for young neuroscientists, October 9th-11th 2024, Verona, Italy. poster presented "*Cortical hypomyelination is associated with cognitive impairment in a mouse model of oligodendroglia-specific deletion of Citron Kinase*" Membership in Scientific Societies in 2024: • Federation of the European Neuroscience Societies (FENS) • Italian Society of Neuroscience (SINS) • Brainstorming Research Assembly for Young Neuroscientists (BraYn) association • Federazione Italiana Scienze della Vita (FISV)
Organizational activities and responsibilities at NICO:	--
Speakers invited:	--
Other organizational activities:	--
Workshops, Schools or Conferences organized:	--
Technology transfer achievements (patents, etc.):	--

Olga Bianciotto, PhD candidate

Supervised PhD students:	na
Honors, prizes, awards:	--
Outreach activities	
• International collaborations:	GiorgiaQuadrato (University of Southern California)
• Invited talks:	--
• Science communication:	• Ricercatori alla Spina – Brain edition, 12th March 2024 • Booth "Exploring the Human Mind" at Notte EuropeadelleRicercatrici e deiRicercatori, Unight 2024, September 28th, 2024
• Editorial duties:	--
• others	Attended courses: • NSAS (Neuroscience School of Advanced Studies) Course: "Neural Stem Cells: Organoids and Human Translation" May 4th-11th, 2024, Venice • Genomica e TecnologieAvanzate - FISV Course, 25th, 26th January 2024 • "Pluripotent Stem Cell On-Demand Training Course" by

	StemCell Technologies, March 2024 Attended PhD courses: • "From neural cell excitability to brain circuit recording, visualization and manipulation" February-March 2024 • "Bibliography and bibliometrics like pros: Stay up to date and Develop Literature Syntheses in the Biomedical Domain (Life and Health Sciences)" March-May 2024 • Workshop: "Public engagement and outreach", 12th December 2024 Attended meetings and workshops: • CSHL Meeting "Glia in Health and Disease" 20-24th August 2024 • D3 4 Health Midterm Conference, 10 Oct 2024. Poster presented: *Optimization of cerebellar organoids for developmental studies and disease modeling* • EMBO Workshop "Unlocking human brain complexity using 3D culture and single-cell omics" 13-16th October 2024, Capri. Poster presented: *Enhancing human cerebellar organoids with enrichment of region-specific astrocytes* • Workshop: "Mental Health in Academia and Virtuous Academic Culture", 17th October 2024 Attended Seminars and webinars: • Seminar cycle (2024) of the PhD in Neuroscience, University of Turin • Webinar: "Organoid meets Microelectrode Array (MEA) – Accelerating Drug Discovery and Development", InsideScientific, 24th January 2024 • NICO NeuroWebinar series International mobility grants obtained: • CIB (Consorzio Interuniversitario Biotecnologie) international mobilitygrant • Company of Biologists' Travelling Fellowship Membership in Scientific Societies in 2024: Italian Glial Network ALBA Network
Organizational activities and responsibilities at NICO:	--
Speakers invited:	--
Other organizational activities:	--
Workshops, Schools or Conferences organized:	--
Technology transfer achievements (patents, etc.):	--

Arianna Contato, Research Fellow

Supervised PhD students:	na

Honors, prizes, awards:	--
Outreach activities	
• International collaborations:	--
• Invited talks:	--
• Science communication:	--
• Editorial duties:	--
• others	Attended seminars and webinars: NICO NeuroWebinar - on fridays, at 2.00 p.m.- https://www.nico.ottolenghi.unito.it/Agenda/NICO-NeuroWebinar Membership in Scientific Societies in 2024: Italian Glial Network
Organizational activities and responsibilities at NICO:	--
Speakers invited:	--
Other organizational activities:	--
Workshops, Schools or Conferences organized:	--
Technology transfer achievements (patents, etc.):	--

Giada Musso, Research Fellow

Supervised PhD students:	na
Honors, prizes, awards:	--
Outreach activities	
• International collaborations:	Prof. Michaela Kress (Medical University Innsbruck), Dr. Franziska Rother (MBC, Berlin)
• Invited talks:	--
• Science communication:	--
• Editorial duties:	--
• others	Attended meetings: Poster Presentation at MND Conference 2024. Christian O. Pritz, Giada Musso, Sofia Dotta, Franziska Rother, Michael Bader, NataliyaOkladnikov and Letizia Marvaldi. Title: Quantifying paw motor function and posture after sciatic nerve injury keypoint segmentation. “Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies” (III edition), Torino, Italy (08-09/11/2024). Attended conference: • 7th International Symposium on Peripheral Nerve Regeneration. Milan, Italy (7-9/05/2024). • Pain mechanism and Therapeutics Conference Verona, Italy (17-22/05/2024).
Organizational activities and responsibilities at NICO:	--
Speakers invited:	--
Other organizational activities:	Member of the local organizing committee at workshop “Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies” (III edition), Torino, Italy (08-09/11/2024)

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

ALL LAB MEMBERS

Activities:	All lab members participate in 'The astrocyte Cafè' and in the 'Italian Glia Network' initiatives

4. Research activity in 2024

a. Summary

In 2024 the outputs of our group primarily consisted in collaborative studies that unveiled new aspects of astrocyte physiopathology. We clarified the cellular mechanisms of the dynamic transition of astrocytes into neurogenic stem cells in the lesioned mouse striatum and contributed to show that Mecp2-KO astrocytes are synaptotoxic to neurons. We also that in the mouse mature brain only in some regions such the subcortical white matter astrocyte turnover is supported by active astrocyte progenitor proliferation.

b. Background and rationale

Our research is centers around two main areas: (i) glial cell physiopathology and (ii) the reconstruction of functional CNS circuits through cell replacement approaches using pluripotent stem cell derived human neurons.

In 2024, our primary focus has been on exploring astrocyte diversity. We investigated the neurogenic astrocyte response to excitotoxic lesion in the mouse striatum and of the occurrence of active astrogliogenesis across different white matter regions in the mature brain. In particular, it was unknown whether the neurogenic activation of striatal astrocytes is a unique property of a defined subset of astrocytes and if this activation is restricted to distinct areas within the lesioned striatum. Additionally, it remained to be assessed whether neurogenic astrocytes exhibit a specific reactive profile that predisposes them to transition toward neurogenesis.
Expanding on this, we also explored cerebellar astrocyte diversity investigating whether the mouse cerebellar white matter serves as a niche for active astrogliogenesis, similar to findings obtained in the subcortical white matter, which seems to support ongoing astrocyte turnover in the mature mouse brain.
In parallel, we examined astrocyte dysfunction, contributing to understanding how astrocyte-specific mutations modeling Rett syndrome influence neuronal connectivity and functionality in murine models.
Exposure to particulate matter (PM) is one of the environmental factors proposed to worsen the disease course of CNS pathologies targeting oligodendroglia, such as Multiple Sclerosis (MS). In 2025, we will finalize two studies aimed at understanding the effects - and the underlying mechanisms - of PM exposure in a mouse model of MS, i.e. Experimental Autoimmune Encephalomyelitis.
In parallel, we have been investigating the maturation in vivo of a second-generation protocol generating human striatal progenitors (Conforti et al Cell Rep Methods 2022) with the aim to improve the maturation and the integration of the striatal progenitor preparation we had investigated before (Schellino et al., Stem Cell Res 2023).

c. Objectives

During 2024 we specifically aimed to:

(i) investigate mature astrocyte diversity within and across brain regions in regard to their capability to activate neurogenic responses and support astrogliogenesis;
(ii) pinpoint pathological actions of diseased astrocytes in neurodevelopmental disorders;
(iii) testing if second-generation human striatal progenitors display an improved maturation and connectivity compared to cells used so far, in vivo upon transplantation into the lesioned striatum.

d. Results

In 2024 we contributed to 9 collaborative publications and submitted two manuscripts, which we are preparing for the resubmission upon revision. Major outputs were:

Regarding astrocyte diversity, in Fogli*, Nato* et al (2024) we published that the neurogenic potential of striatal astrocytes in the mouse adult striatum upon excitotoxic lesion is widespread and not restricted to specific cell subsets. Activation occurs at the lesion border, where it associates with different types of astrocytes reactivity. Further, the continuous stochastic activation of local astrocytes supports steady state neurogenesis. This work is the result of our long-standing NICO collaboration with F. Luzzati and his team.

In Bocchi et al. (2024) we contributed to compare mouse astrocytes of the white matter with those of the cerebellum. While white matter astrocytes undergo proliferation and maintain a progenitor pool supplying new astrocytes to the cerebral cortex grey matter, cerebellar white matter astrocytes do not undergo proliferation. It seems therefore that the turnover of astrocytes is not the same across brain regions. Of note, no astrogliogenesis was found in the human subcortical white matter pointing to species specific differences.

In Albizzati et al (2024) we contributed technical support used to demonstrate that Mecp2KO astrocytes secretes IL6 which negatively affects neuronal synapses. This contribution set the basis for the current collaboration with A Frasca (University of Milan).

In Pallavicini et al (2024) we focused on hereditary microcephaly due to CIT-K mutations and characterized mouse mutant where the CitK kinase domain is disrupted. These showed a mild phenotype that did not phenocopy microcephaly, otherwise found in humans with corresponding mutations and modeled in organoids. These results underscore species differences in CitK role in corticogenesis. Also in the frame of our collaboration with Prof. F Di Cunto's group at NICO, in Iegiani et al. (under consideration, Cell Death and Disease, ID:CDDIS-25-0042-T), we contributed to investigate the functional interactors of CIT-K in DNA damage repair.

Finally, in Bonato et al., (Under revision, Eur J Neurosci, ID: EJN-2024-09-32062), we tested the hypothesis that exposure to PM_{10} might influence the disease course and severity in the experimental autoimmune encephalomyelitis (EAE) mouse model of MS. We found a selective vulnerability of immunologically primed mice toward the effects of PM_{10}, occurring before the emergence of overt motor impairment and presenting as specific behavioral alterations.

e. Advancement in the field

These findings

- change the notion about the neurogenic competence of mature parenchymal astrocytes demonstrating that is widespread and that upon activation it matches that of SVZ astrocytes for a number of features (Fogli, Nato et al 2024);
- suggest that astrocyte turnover may constitute a form of region-specific plasticity (Bocchi et al., 2024);
- show that astrocyte-derived IL6 participates in synaptic defects found in Rett syndrome models and therefore constitutes a therapeutic target for the Rett syndrome (Albizzati et al. 2024).

f. Publications

Digiovanni S, **Lorenzati M**, **Bianciotto OT**, Godel M, Fontana S, Akman M, Costamagna C, Couraud PO, **Buffo A**, Kopecka J, Riganti C, Salaroglio IC. Blood-brain barrier permeability increases with the differentiation of glioblastoma cells in vitro. Fluids Barriers CNS. 2024 Nov 1;21(1):89.
Research article Q1, Scopus, Developmental Neuroscience

Garello F, Cavallari E, Capozza M, **Ribodino M**, Parolisi R, **Buffo A**, Terreno E. MRI detection of free-contrast agent nanoparticles. MagnReson Med. 2025 Feb;93(2):761-774.
Research article Q1, Scopus, Radiology, Nuclear Medicine and Imaging

Pallavicini G, Moccia A, Iegiani G, Parolisi R, Peirent ER, Berto GE, **Lorenzati M**, Tshuva RY, Ferraro A, Balzac F, Turco E, Salvi SU, Myklebust HF, Wang S, Eisenberg J, Chitale M, Girgla NS, **Boda E**, Reiner O, **Buffo A**, Di Cunto F, Bielas SL. Modeling primary microcephaly with human brain organoids reveals fundamental roles of CIT kinase activity. J Clin Invest. 2024 Nov 1;134(21):e175435.
Research article Q1, Scopus, General Medicine

Fogli M,* **Nato G***, Greulich P, Pinto J, **Ribodino M**, Valsania G, Peretto P, **Buffo A**, Luzzati F. Dynamic spatiotemporal activation of a pervasive neurogenic competence in striatal astrocytes supports continuous neurogenesis following injury. Stem Cell Reports. 2024 Oct 8;19(10):1432-1450.
Research article Q1, Scopus, Biochemistry, Genetics and Molecular Biology

Marangon D, Castro E Silva JH, **Cerrato V**, **Boda E**, Lecca D. OligodendrocyteProgenitors in GlialScar: A Bet on Remyelination. Cells. 2024 Jun 12;13(12):1024.
Literature Review Q1, Scopus, Biochemistry, Genetics and Molecular Biology

Ghibaudi M, **Boda E**, Bonfanti L. From mice to humans: a need for comparable results in mammalian neuroplasticity. Neural Regen Res. 2025 Feb 1;20(2):464-466. doi: 10.4103/NRR.NRR-D-24-00143. Epub 2024 Apr 16.
Literature Review Q1, Scopus, Neuroscience (miscellaneous)

Albizzati E, Breccia M, Florio E, Cabasino C, Postogna FM, Grassi R, **Boda E**, Battaglia C, De Palma C, De Quattro C, Pozzi D, Landsberger N, Frasca A. Mecp2 knock-out astrocytesaffectsynaptogenesis by interleukin 6 dependentmechanisms. iScience. 2024 Feb 23;27(3):109296.
Research article Q1, Scopus, Multidisciplinary

Decoster L, Trova S, Zucca S, Bulk J, Gouveia A, Ternier G, Lhomme T, Legrand A, Gallet S, Boehm U, Wyatt A, Wahl V, Wartenberg P, Hrabovszky E, Rácz G, Luzzati F, **Nato G**, Fogli M, Peretto P, Schriever SC, Bernecker M, Pfluger PT, Steculorum SM, Bovetti S, Rasika S, Prevot V, Silva MSB, Giacobini P. A GnRH neuronal population in the olfactory bulb translates socially relevant odors into reproductive behavior in male mice. Nat Neurosci. 2024 Sep;27(9):1758-1773.
Research article Q1, Scopus, Neuroscience/General Neuroscience

Bocchi R., Thorwirth M, Simon T, Koupourtidou C, Clavreul S, Della Vecchia P., Bottes S., Jessberger S., Zhou J, Wani G, Pilz GA, Ninkovic J, **Buffo A**, Sirko S, Götz M and Fischer J Region-specific astrocyte heterogeneity in the white matter reveals region-specific astrogenesis Nature Neurosci 2024
Research article Q1, Scopus, Neuroscience/general Neuroscience

* co-first author

5. Future directions and objectives for next years

a. Summary

In 2025 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 launch a new project specifically targeting the reconstruction of striatal circuits, with a focus on understanding their functional implications. This work aims to address critical gaps in the restoration of complex neural networks.

Our investigation of astrocyte heterogeneity will expand to include human cerebellar samples and models, such as cerebellar organoids. Moreover, we will explore the contribution of astrocytes to Rett syndrome by modeling glia-neuron interactions using human cells, aiming to uncover and treat novel insights into the disease's underlying mechanisms.

In parallel, we will investigate how oligodendroglial signaling molecules, environmental pollutants, and therapeutic strategies affect myelin formation, maintenance, and regeneration. Specifically, we will examine the effects of exposure to particulate matter on the progression of disease in a mouse model of Multiple Sclerosis and will evaluate the therapeutic potential of non-invasive brain stimulation in a mouse model of myelin injury. In addition, we will assess pharmacological approaches targeting oxidative stress in cases of developmental hypomyelination.

b. Background and Significance

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 regards a diffuse projection system. When it comes to the reconstruction of more complex circuits such as those damaged in Huntington's disease, information is scarce. In particular, critical questions remain unresolved, including:
-the feasibility of comprehensive reconstruction of the direct and indirect pathways;
-the extent to which accurate circuit reconstruction contributes to the restoration of lost functions in a diseased brain;
-the specific role of graft activity in driving the behavioral improvements observed following grafting.
These unresolved aspects form the basis of our ongoing research efforts.

Glial physiopathology

Glial cells are now recognized as 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 found molecular diversity across and within main AS adult types (in preparation). This extends the canonical classification and points to functionally specialized AS

subtypes which emerge from two largely distinct maturational trajectories likely originated by two molecularly distinct progenitor pools. Astrocyte types and their development remain to be investigated in the human cerebellum.

Furthermore, recent evidence has shown that Mecp2 deficiency in mouse astrocytes impairs their ability to support synapse formation by releasing synaptotoxic molecules (Albizzati et al., 2024). However, the relevance of this mechanism to human pathology remains to be established. Investigating these aspects in human models will be crucial for understanding the implications of astrocyte dysfunction in disease and for identifying potential therapeutic strategies.

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 developmental diseases including the MCPH17 microcephaly. Yet, the mechanistic aspects of these pathologies are largely unknown, and therapies are lacking. 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

In 2025, we will work toward these main general aims:
- expand the study of adult circuit rewiring through cell replacement approaches, focusing on factors and mechanisms supporting the reconstruction of the complexity of basal ganglia circuits;
- investigate the role of correct myelin deposition and plasticity in cognitive functions;
- in the study of glial cells, we plan to focus on human astrocytes and examine the mechanisms governing the interplay between astrocytes and neurons in physiology and in neurodevelopmental diseases.

In detail we contribute to the mission of NICO by: (i) studying the plasticity of the developing, healthy and diseased brain; (ii) expanding the knowledge on fundamental processes of glial cell physiopathology; (ii) exploring preclinical therapeutic approaches for demyelinating and neurodevelopmental disorders; (iii) developing innovative humanized experimental models and analytical approaches.

d. **Specific objectives and strategies**

Brain plasticity and repair

Reconstruction of functional CNS circuits through cell replacement

In 2025 we will complete the investigation on the integration, functionality and therapeutic action of second-generation human striatal grafts (Conforti et al. 2022) and we will reorganize our team and activities within this research line thanks to the award of the Synergy Grant CUSTOM_MADE. In this grant we will focus on mapping and promoting correct circuits by the grafted cells and on devising of cell preparations where graft activity can be increased or decreased in defined graft neuron subsets with the aim to achieve functional rescue.
Coll: Prof E Cattaneo, Univ Milano; M. Parmar, Univ Lund; J. Emneus, Technical University Copenhagen.

Myelin plasticity and regeneration

Enhancing neuronal activity has been shown to promote oligodendrogenesis and remyelination in preclinical studies. Thus, in 2025, we will investigate the suitability of a non-invasive brain stimulation technique (i.e. transcranial direct current stimulation - tDCS) as an option to boost myelin repair and plasticity in a mouse model of myelin injury. Collaboration with Dr. M Cambiaghi, Univ of Verona.

Along this line, we also exploit mouse models of dysmyelination to study the contribution of myelin and myelin remodeling in the cross-talk between cortical and subcortical brain areas as a substrate of cognitive functions.

Glia physiopathology

Study of astrocyte heterogeneity

Results on the heterogeneity of mature astrocytes in the mouse cerebellum and its developmental origins are being assembled into a manuscript entitled "A Single-Cell Atlas to Unveil the Diversity of Mouse Cerebellar Astrocytes". Collaboration with Prof. L. Telley (University of Lausanne) and Prof. M. Götz (LMU). Ongoing focus is on the validation of specific gene expression patterns, on the interactions between specific astrocyte subtypes and cerebellar neurons and on addressing astrocyte diversity to fetal cerebellar samples.
In parallel, we are modeling human astrogliogenesis in vitro by generating regionalized glial progenitors and differentiated glial cells from hPSCs. This includes crafting cerebellar organoids spiked with preformed glia and assembling human cerebellar organoids with gliospheres to create advanced assembloids. Coll: Dr. G. Quadrato, USC.

Understanding astrocyte physiopathology

We will extend the study of the pathogenic actions of astrocytes carrying Mecp2 mutation by studying the interplay between astrocytes and neurons derived from hiPSC of RETT patients and isogenic controls. Collaboration with A. Frasca (University of Milano) and O. Brustle (University of Bonn).

Limiting damage in demyelinating or neurodevelopmental disorders

In a recently funded project (UNFOLD) we proposed to develop an innovative fully humanized in vitro model to study Multiple Sclerosis and perform drug screening. We started setting up an integrated rodent 2D system recapitulating tissue complexity and inflammatory demyelination which will be implemented in the human integrated system. L. Marvaldi and G. Gambarotta (NICO) also participate in this Grant.
Epidemiological and experimental studies – including our own (see above) - strongly suggest that people with MS (or predisposed to develop MS) are a population cohort vulnerable to the effects of exposure to PM. The biological substrate of such vulnerability is unknown. In animal models, we'll investigate whether individuals primed to develop autoimmunity against CNS myelin (as it occurs in MS) respond differently to PMcompared to healthy subjects, by focusing on plasmatic extracellular vesicles (EVs) and their miRNA cargo. Collaboration with Prof. L Ferrari and V Bollati (Univ of Milan), Dr. F Montarolo (NICO).
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), Prof. M. Boido, Prof. F. Di Cunto (NICO), Prof. C. Riganti (Dept of Oncology, MBC, Univ of Turin).

e. Unique features of the project research

Our research stands out in several key ways:
- innovative perspective on glial cells: we leverage the originality of the angle on glia cells that is unique to our research context;
- use of human cell models: by leveraging the power of human cell models, we increase the depth and relevance of our investigations;
- integration of advanced methodologies: our studies combine multilevel methodological approaches with cutting-edge techniques, providing both novelty and methodological rigor;
- we ask fundamental, unanswered research question by using original models and approaches:

Key Research Questions and Objectives:
Plasticity of the diseased mature brain: to what extent can complex circuits be reconstructed in the adult diseased brain by the addition of new healthy neurons? To what extent is anatomical reconstruction needed to support functional circuits? We address this topic using the basal ganglia circuits as a model, and human cells and platforms where human circuits can be studied.

Physiological plasticity: we work in the emerging field of myelin plasticity, an adaptive mechanism whose driving factors and modes of action remain poorly understood. Our work aims to illuminate this largely unexplored area.

Glial cell physiopathology: we seek to unravel the mechanisms of glial cell specification and maturation, as well as their complex interactions with neurons in both healthy and pathological states. These aspects are particularly crucial for gaining a complete understanding of brain development, function and dysfunction. We add elements of originality by focusing on the rodent and human cerebellum and its specific glial and neuronal populations as models and now we further enhance our studies by integrating a human organoid model of the cerebellum.

f. **Methodology**

In the past, we have invested in integrating state-of-the-art technologies and advanced analytical tools to elevate the quality and multilevel comprehensiveness of our research. Omics technologies, human in vitro models and sophisticated in vivo manipulations are currently used in the ongoing projects.
Additional innovations developed in 2024 include:

- Optimization of human cerebellar organoids with glial cell enrichment
- Development of a computational multimodal pipeline for maturational trajectory inferences in complex datasets
- Development of a computational pipeline for miRNA/miRNA target network and gene-to-disease analyses.

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 2024

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
Pritz, Christian, Tenure Track Researcher, 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, Post doctoral fellow, PhD in Complex Systems for Quantitative Biomedicine, 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.

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).

Greco, Flavia, PhD Student, MSc in Cellular and Molecular Biology, 09/06/1998, 011 6706616, flavia.greco@unito.it, Generation and analysis of C. elegans models of brain diseases.

Ghibaudo, Alessia, Research Fellow, MSc in Cellular and Molecular Biology, 19/01/1999, 011 6706616, alessia.ghibaudo@unito.it, Generation and analysis of C. elegans models of brain diseases.

Lazzerini, Letizia, Research Fellow 'Fondazione Venesio', MSc in Molecular and Applied Biology, 18/06/1998, 011 6706616, lazzerini.letizia08@gmail.com, in vitro and in vivo analysis of neuronal cells

2. CURRENT GRANTS

Starting-end date	Project Title and ID	Beneficiary	Funding Program/Agency	Role of the unit	Overall AmountFunded	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
28/09/2023 - 27/09/2025	Dissection of common mechanisms in genetic	PI	PRIN 2022 funded by EU through Next Generation	Coordinator	72289	UNITO

	primary microcephaly. 2022M75NN8		EU program.			
26/09/2024 - 25/09/2026	IDH1/2 mutation inhibition in lower grade gliomas: from preclinical models to clinical applications PNRR-TR1-2023-12378219	PI	Next Generation EU PNRR 2023	Unit leader	175000	FCO

3. SCIENTIFIC ACTIVITIES IN 2024

Name, Role (PI)

Supervised PhD students:	Direct supervisor of: Alessia Ferraro and Flavia Greco Co-supervisor of: Gianna Pavarino, Vanessa Chiappini e Sofia Dotta.
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:	18-03-2024: BIEVOL Associatn (www.bievol.org) - XV BIOETHICS WEEK - Speech entitled: Comparison between different types of natural intelligence and artificial intelligence 12-10-2024: INNOVATION AND SCIENCE FESTIVAL - SettimoTorinese – Talk ‘The nervous system in deep space’ 28-09-2024: UNIGHT – European Researchers' Night 2024– Talk ‘The nervous system in deep space’
• 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 - Responsible for Corruption Prevention and Transparency
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Alessia Ferraro, PhD student

Supervised PhD students:	
Honors, prizes, awards:	ABCD-SIBBM National PhD meeting – Bologna 25-27/03/2024 – Travel grant winner
Outreach activities	
• International collaborations:	
• Invited talks:	ABCD-SIBBM National PhD meeting – Bologna 25-27/03/2024 – Oral Presentation: "CITK catalytic activity inhibition through Lestaurtinib leads to DNA damage, cytokinesis failure and cell death in brain tumors" The Neuroscience of Cancer meeting - Bologna 2-4/10/2024 - Oral Presentation: "Targeting citron kinase catalytic activity for high grade brain tumor treatment"
• 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.):	

4. Research activity in 2024

a. Summary

We study the genetic and molecular mechanisms that control neuron generation, survival and differentiation during normal brain development and how the alteration of these processes may lead to neurodevelopmental disorders, 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

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 or 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, we have been studying for many years the neurological syndrome produced 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. Our latest achievement was to highlight the relevance of human brain organoids in the study of human-specific mechanisms of primary microcephaly, which cannot be adequately represented in rodent models. Using this assay, we showed that the catalytic activity of CITK is much more essential in human than in mouse development, paving the way for further studies on the relevan cell biology. 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, with the aim of developing new specific drugs for these devastating tumors.

c. Objectives

Our constant research aims 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 and microtubule dynamics;
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

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. We have recently shown that DNA damage, apoptosis and cytokinesis failure produced by CITK loss are strongly dependent on the presence of the non-kinase domains of CITK, while the catalytic activity seems specifically necessary for polarized cytokinesis in developing human brain organoids.

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. Recently, we have found that another important link between CITK and microtubule may be represented by the HDAC6 proteins, whose inhibition can rescue microcephaly in a zebrafish model of MCPH17.

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, as wella as of MCPH17.

e. Advancement in the field

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 is expected to provide major contribution to the molecular and cellular understanding of disease mechanisms in a wide variety of neurodevelopmental disorders.

f. Publications

Pallavicini G, Moccia A, Iegiani G, Parolisi R, Peirent ER, Berto GE, Lorenzati M, Tshuva RY, Ferraro A, Balzac F, Turco E, Salvi SU, Myklebust HF, Wang S, Eisenberg J, Chitale M, Girgla NS, Boda E, Reiner O, Buffo A, Di Cunto, F, Bielas SL. 2024 Modeling primary microcephaly with human brain organoids reveals fundamental roles of CIT kinase activity. J Clin Invest. Nov 1;134(21):e175435.
Research article Q1

Musso G, Dotta S, Parmar A, Rasà DM, Di Cunto F, Marvaldi L. 2024 Standardization of a Novel Semi-Automatic Software for Neurite Outgrowth Measurement. J Vis Exp. Aug 9;(210).
Research article Q3

5. Future directions and objectives for next years

a. Summary

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

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

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

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. To this regard, we are currently investigating the interplay between CITK, CYLD and HDAC6, since the latter proteins are connected both to the regulation of microtubule stability and to DNA repair.
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

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

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 have partially setup this system at NICO, and we plan 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. the development of the genetically tractable model *C. elegans* among the main platforms of the Institute.

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2024

Laboratory name: **Neuropsychopharmacology**

1. LABORATORY DESCRIPTION – PERSONNEL:

Principal Investigator
Surname: Bertocchi, name: Ilaria, position: research assistant (RTD-B), degree: PhD, birthdate: 13/04/1982, phone: +39 0116706611, email: ilaria.bertocchi@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, laboratory rodents

Surname: Cifarelli, name: Lorenzo, position: PhD student, degree: medical biotechnology, birthdate: 17/07/1997, phone: +39 0116706632, email: lorenzo.cifarelli@unito.it, role & expertise: conduction of experiments with expertise in handling murine models of neuropsychiatric disorders, behavioral tests, epifluorescence microscopy, image analysis, data analysis

Surname: Lodi, name: Alessandra, position: research fellow, degree: Neuroscience, birthdate:18/01/1994, phone: +39 0116706611, email: alessandra.lodi@unito.it, role & expertise: conduction of experiments with expertise in molecular and cellular biology, immunohistochemistry, laboratory rodents and flies, behavioral tests, image and data analysis

2. CURRENT GRANTS

Starting-end date	Project Title and ID	Beneficiary	Funding Program/Agency	Role of the unit	Overall AmountFunded	Managed by FCO/UNITO
2023-2025	Matrix Metalloproteinase-9 and PeriNeuronal Nets: new therapeutic targets for Fragile X Syndrome	Carola Eva	PRIN: PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE	ex-PI	204298	UNITO
2025-30/6/2027	Progetto Dieta e Salute: impatto della dieta chetogenica nel trattamento dell'epilessia farmacoresistente	Ilaria Bertocchi	Fondazione CRT	PI	38000	UNITO

3. SCIENTIFIC ACTIVITIES IN 2024

Ilaria Bertocchi, Research assistant (RTD-B)_PI

Supervised PhD students:	Lorenzo Cifarelli
Honors, prizes, awards:	n/a
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, New York, NY, USA -Daniela Carulli, Laboratory for Neuroregeneration Netherlands Institute for Neuroscience (NIN) Meibergdreef 47 - 1105 BA Amsterdam - The Netherland
• Invited talks:	'Dietary manipulations and their impact on both peripheral and central inflammation: friend or foe?' Satellite International Workshop 'Obesity and eating disorders' Camerino, 7-8 June 2024
• Science communication:	'Radiprodil, a selective GluN2B negative allosteric modulator, rescues audiogenic seizures in mice carrying the GluN2A (N615S) mutation' GRI Genes Research Roundtable meeting, Thursday, June 6, 2024, from 12:30pm EST- 2:00pm EST. CureGRIN 'Le reti perineuronali e il loro ruolo nei disordini neuropsichiatrici' PINT OF SCIENCE FESTIVAL, 13-15 Maggio 2024
• Editorial duties:	Reviewer for the following journals (2024): Frontiers in pharmacology Frontiers in immunology
• others	Oralpresentationat the 42° Congresso Nazionale della Società Italiana di Farmacologia, Sorrento. Title of the talk: 'Testing the antiseizure effect of NMDA inhibitors in mice carrying a Gain of Function mutation (N615S) in the GluN2A subunit of the NMDA receptor' Thursday, November 14th, 2024 Poster presentationat the 42° Congresso Nazionale della Società Italiana di Farmacologia, Sorrento. Title: 'Effects of a ketogenic diet in a murine model of GRIN2A syndrome' Attended Congress: 6th European GRIN Conference Saturday, November 9 and Sunday, November 10 2024 Attendedcourses: Corso di Formazione Generale alla Salute e Sicurezza per i Lavoratori (art. 37 del D.Lgs. 81/08) IRIDI START: Insegnamento di qualità in presenza e a distanza, valutazione e inclusione
Organizational activities and responsibilities at NICO:	In charge for behavioral labs I and II (animal facility)
Speakers invited:	-Ferdinand Althammer (Universitätsklinikum Heidelberg) 15/03/2024 -Gabriele Chelini, CNR Pisa, 20/12/2024
Other organizational activities:	-'The Science Bridge' advisory board member https://thesciencebridge.org/ -Comitato scientifico assmgrin2aitalia https://assmgrin2aitalia.it/comitato-scientifico/ -Comitato scientifico di associazione GRI Italia https://www.gri-italia.it/

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

Alessandra Oberto, Research associate (RU)

Supervised PhD students:	Lorenzo Cifarelli
Honors, prizes, awards:	n/a
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 -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, New York, NY, USA -Daniela Carulli, Laboratory for Neuroregeneration Netherlands Institute for Neuroscience (NIN) Meibergdreef 47 - 1105 BA Amsterdam - The Netherland
• Invited talks:	n/a
• Science communication:	n/a
• Editorial duties:	n/a
• others	Poster presentationat the 42° Congresso Nazionale della Società Italiana di Farmacologia, Sorrento. Title: 'NPY-Y1R signal, gonadal hormones and diet interaction in modulating neuroplasticity and inflammation in female mice'
Organizational activities and responsibilities at NICO:	In charge for behavioral labs I and II (animal facility)
Speakers invited:	n/a
Other organizational activities:	n/a
Workshops, Schools or Conferences organized:	n/a
Technology transfer achievements (patents, etc.):	n/a

Lorenzo Cifarelli, PhD Student

Supervised PhD students:	n/a
Honors, prizes, awards:	SIF Scholarship for best oralcommunication, 42° congresso nazionale della SIF (Società Italiana di Farmacologia) (Sorrento, 13-16/11/2024)
Outreach activities	
• International collaborations:	n/a
• Invited talks:	n/a
• Science communication:	n/a
• Editorial duties:	n/a
• others	Poster presentation at ConvegnoMonotematico SIF (SocietàItaliana di Farmacologia) "Behavioral and metabolic aspects of obesity and eating disorders" (Camerino, 06-7/06/2024). Title: Study of Behavior, Plasticity-related Markers and Neuroinflammation in a Mouse Model of Developmental and Epileptic Encephalopathy following a Ketogenic Diet

	Poster presentation at FENS (Federation of European Neuroscience Societies) forum 2024 (Vienna, 25-29/06/2024). Title: Whole-Brain Perineuronal Net and Parvalbumin Expression Analysis in Fragile X Syndrome Mice Poster presentation at 7th BraYn (Brainstorming Research Assembly for Young Neuroscientists) conference 2024 (Verona, 9-11/10/2024). Title: Ketogenic Diet in the GluN2A(N615S) Mouse Model Improves Behavioral Impairments in a Sex-based Manner, Rescues Audiogenic Seizures and Affects Neuronal Plasticity and Neuroinflammation Oralpresentationat 42° congresso nazionale della SIF (Società Italiana di Farmacologia) (Sorrento, 13-16/11/2024). Title: Perineuronal net and parvalbumin expression analysis in fragile X syndrome mice at different developmental stages
Organizational activities and responsibilities at NICO:	n/a
Speakers invited:	n/a
Other organizational activities:	n/a
Workshops, Schools or Conferences organized:	n/a
Technology transfer achievements (patents, etc.):	n/a

Alessandra Lodi, Research Fellow

Supervised PhD students:	n/a
Honors, prizes, awards:	n/a
Outreach activities	
• International collaborations:	n/a
• Invited talks:	n/a
• Science communication:	n/a
• Editorial duties:	n/a
• others	Poster presentation at the Monotematic SIF Congress "Behavioral and metabolic aspects of obesity and eating disorder", Camerino. Title: "NPY-Y1R signal, gonadal hormones and diet interaction in modulating neuroplasticity and inflammation in female mice" Poster presentation at the 7thBraYn Conference, Verona. Title: "Impact of Genotype on Maternal Care in a mouse model of Fragile X Syndrome" Poster presentationat the 42° Congresso Nazionale della Società Italiana di Farmacologia, Sorrento. Title: "Impaired Maternal Behavior in Fragile X syndrome Mutant Mice: potential implications"
Organizational activities and responsibilities at NICO:	n/a
Speakers invited:	n/a
Other organizational activities:	n/a
Workshops, Schools or Conferences organized:	n/a
Technology transfer achievements	n/a

(patents, etc.):	

4. Research activity in 2024

a. Summary

We started a project to investigate the mechanisms through which a ketogenic diet may impact seizure susceptibility and cognitive deficits in a mouse model of a *GRIN2A*-related syndrome (DEE). Our second goal was to conclude an old chapter of our research by studying the link between NPY-Y1R, gonadal hormones and diet in modulating inflammation, behavior and plasticity. Finally, we are carrying on parallel studies using the Fmr1KO model: i) the impact of maternal behavior of Fmr1KO mice on offspring and ii) the role of perineuronal nets in the pathogenesis of fragile X syndrome.

b. Background and rationale

1. Epileptic and developmental encephalopathies (DEEs) are disorders that include a large and heterogeneous group of epileptic syndromes that arise in childhood and for which there is no cure. DEEs of genetic origin (30-50%) are mainly due to sporadic de novo mutations that affect genes involved in the control of neuronal excitability, such as GRIN genes, encoding for the different subunits of the NMDA receptors (Bertocchi et al., 2021, 2023, 2024). It has been suggested that a set of genetic, environmental and biological factors (including metabolic and neuronal inflammation) increase the susceptibility to seizures and cognitive impairments observed in patients affected by DEE.
Ketogenic diet (KD) is a dietary protocol documented to be highly effective for children and adults with drug resistant epilepsy. Furthermore, several studies suggest a strengthening effect on cognitive abilities and benefits from a metabolic and physiological point of view. However, only few preclinical and clinical studies have analyzed the effects of KD, and the results have not been conclusive. Discordant data also exist regarding inflammation and the effects produced by KD at a peripheral level. This diet is therefore poorly integrated into clinical practice, mainly due to scarce knowledge on: (i) biomolecular mechanisms, (ii) specific biomarkers of efficacy/toxicity, (iii) risks deriving from prolonged use. It is essential to have new experimental data to improve the level of knowledge on KD.
2. We demonstrated that female gonadal hormones and the hypothalamic NPY-Y1R system functionally interact in regulating neuroinflammation and susceptibility to obesity and associated disorders (Oberto et al., 2022). To deepen the knowledge of this interaction is important to include the analysis of behavior and the expression of plasticity markers in our established mouse model (Bertocchi et al., 2011, 2020).
3. Fragile X syndrome (FXS) is the most common monogenic cause of inherited intellectual disability and autism. FMRP protein, which is absent in FXS patients due to a mutation in the Fmr1 gene, 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 failed to show clinical benefit. Fmr1KO mice represent a solid and useful model to study the effects of the lack of FMRP. In addition to genetic influences, environmental factors play a critical role in shaping the behavioral phenotype in FXS. Namely, adverse environments, including maternal stress and insufficient caregiving, can exacerbate neurodevelopmental and behavioral symptoms (Bertocchi et al., 2011). Moreover, an impairment of perineuronal nets (PNN) formation around GABAergic parvalbumin-positive neurons in the developing auditory cortex (AC) of Fmr1KO mice has been reported. PNNs are specialized forms of extracellular matrix, which arise in concomitance with the closure of developmental critical periods of plasticity and control synaptic connectivity and functions in the adult brain (Bertocchi et al., 2021).
Pharmacological or genetic restoration of PNNs in the AC significantly ameliorated FXS-associated hyperresponsivity to acoustic stimuli. However, the role of PNN in other areas of the FXS brains is still

largely unknown. Understanding the impact of impaired caregiving on FXS phenotype and the role of PNN in the whole FXS brain is crucial for identifying novel therapeutic strategies.

c. Objectives

First, we aim to validate the therapeutic efficacy of KD in counteracting seizures and cognitive deficits in a preclinical model of GRIN2A syndrome, documenting protective and/or toxic effects on the brain and peripheral organs. This may be fundamental for the development of new drugs, which facilitate obtaining the same beneficial results induced by KD, but without the problem of adherence to therapy ('compliance') and side effects induced by its chronic intake, therefore more accessible and manageable for everyone.
Second, we want to conclude a long chapter related to sex-dependent modulation of the NPY-Y1R system in our established mouse model of metabolic syndrome.
Third, we want to study PNN distribution in the whole FXS brain, to fill the lack of information related to this topic, and to follow the PNN formation process at different times of postnatal development in brain nuclei important for cognitive and emotional functions of Fmr1KO mice. Moreover, we investigated maternal behavior of Fmr1KO mice and its impact on offspring, aiming to bridge the gap between genetic risk factors and environmental contributions to FXS symptomatology.

d. Results

1- We collected an initial body of preliminary data (more mice are required to get statistical significance) 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 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. 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. Our results are encouraging in some respects and we used them as preliminary data in a funding request which was accepted by Fondazione CRT.
2- We demonstrated that the interplay between gonadal hormones and NPY is crucial for modulating anxiety-like behavior. Moreover, high fat diet (HFD) exacerbated deficits in executive function induced by Y1R conditional knockout and ovariectomy (ovx). NPY/estrogen interplay also affected neuroinflammation in the hippocampus and an additional effect of HFD was visible in the CA1 field. Finally, ovx determined an overall reduction in PNN density and coverage around PV+ neurons particularly in the hippocampus. Data are ready to be published and the manuscript in preparation.
3- We subjected an adequate number of mice at different stages of development (P20, P40 and P60) to a behavioral test battery and we are currently completing the analysis of their brains for PNN and PVI distribution. In this way, we will obtain information about 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. In parallel, we are finalizing the analysis of data and the writing of an important manuscript about a whole brain parvalbumin and perineuronal net expression analysis in Fragile X mice, by Luzzati and Cifarelli et al. We have conducted an automated analysis of WFA+ PNN and PV+ cells in the entire Fmr1KO mouse brain at P60 and found a general reduction of PNNs

and PV in Fmr1KO brains compared to WT ones. Cortex and hippocampus were the brain areas most affected by PNN significant reduction whereas PV+ cell density was significantly decreased in the amygdala.
Additionally, we obtained new data about the effect of maternal care of FXS dams on pups behaviour as adults. We documented differences in maternal behaviour of FXS and WT C57BL/ 6J dams toward their pups during the first postnatal week of life, with Fmr1KO mothers being less caring than WT controls.
Interestingly, we also showed that Fmr1KO +/- mothers are less maternal and more hyperactive compared to the homozygous ones. Pups raised by Fmr1 KO +/- showed reduced sociability, short-term memory and anxiety. This work is ready to be written and published: the scientific community must take these differences into consideration when planning projects involving the use of this important model of the pathology.
4- Lastly, we started a nice collaboration with a research group from the molecular biotechnology center (MBC, Turin). We are currently participating in the drafting of a manuscript that is already under review. The paper is about a new mouse model knock-out for a specific component of the postsynaptic density, which present different plasticity anomalies and an autistic-like phenotype.

e. Advancement in the field

GRIN-related DEEs and FXS are rare syndromes that currently lack effective treatments. Notably, our work demonstrated the high predictive value of our GRIN2A mouse model, as the compound we studied in one of our recent publication (Bertocchi et al., 2024) is currently in use in an ongoing clinical trial in several European centers, and is having excellent results on children affected by these serious rare syndromes (EudraCT Number: 2022-000317-14).
Moreover, our results provided insights into the basic mechanisms of disorders that are common to other neurological conditions as well (e.g hyperexcitability, seizures, cognitive deficits).
Besides, our long-lasting collaboration with Prof. Hasan on active and parallel projects regarding the role of NMDA receptors in learning and memory functions, has led to new evidence about the role of the dentate gyrus in memory retrieval (Carretero-Guillén et al., 2024).

f. Publications

Bertocchi I*, Cifarelli L, **Oberto A**, **Eva C**, Sprengel R, Mirza NR, Muglia P (2024).*Radiprodil, a selective GluN2B negative allosteric modulator, rescues audiogenic seizures in mice carrying the GluN2A(N615S) mutation.* Br J Pharmacol. 2024 Jun;181(12):1886-1894. doi: 10.1111/bph.16361. Epub 2024 Mar 26. PMID: 38529699.
Research article Q1. IF: 9.473

Carretero-Guillén A, Treviño M, Gómez-Climent MA, Dogbevia GK, **Bertocchi I**, Sprengel R, Larkum ME, Vlachos A, Gruart A, Delgado-García JM & Hasan MT (2024). *Dentate gyrus is needed for memory retrieval.*Mol Psychiatry. 2024 Apr 12. doi: 10.1038/s41380-024-02546-0. Epub ahead of print. PMID: 38609585.
Research article Q1. IF: 13.44

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

1) We will complete the newly funded preclinical study on the therapeutic efficacy of KD in counteracting cognitive and behavioral deficits and epileptic seizures typical of early-onset epileptic and developmental encephalopathies (DEE) induced by mutations in the GRIN genes, coding for glutamate receptors.
This is fundamental for developing new drugs with an overall efficacy while avoiding side effects as much as possible. Interestingly, we revealeda sex-dependent difference in AGS susceptibility and in the dose-dependent rescue effect of ASM as well in our homozygous Grin2a$^{S/S}$ mice (Bertocchi et al., 2024). Particularly interesting will be an in-depth study of the causes of the sex-dependent effects observed.
2) Another goal is to validate PNN and MMP-9 as new molecular targets for therapeutic intervention in FXS. We plan to preclinically assess new treatments at the most effective developmental stage, an experimental opportunity impossible to achieve with clinical studies. To do this, we have established a collaboration with NICO members and with national and international experts in the field.
Our desire is to apply at national and international calls for grants on rare diseases.
3) Moreover, we currently have a paper under review about astrocyte-NMDAR-dependent gliotransmission. We have conducted new experiments and collected new evidence related to communication dynamics at the level of the tripartite synapse and we would like to finally publish our revised work. In the meantime, always in collaboration with Prof. Hasan (Achucarro Basque Center for Neuroscience), we have in mind new projects related to the topic and we would also like to involve other NICO members.
4) Lastly, we have planned new experiments to be conducted on a murine model of autism in collaboration with the laboratory of Prof. Defilippi, molecular biotechnology center (MBC).

b. Background and Significance

1) Our project aims to achieve a better understanding of the molecular mechanisms through which KD is able to improve susceptibility to seizures and cognitive deficits. Furthermore, we want to study its impact on a peripheral and physiological level in general. This may be fundamental for the development of new drugs, which facilitate obtaining the same beneficial results induced by this therapeutic regimen, but without the problem of adherence to therapy ('compliance') and side effects induced by the chronic intake of a KD (Bertocchi et al., 2023), and therefore more accessible and manageable for everyone. In fact, there is a need to find new pharmacological approaches that can replace KD, intervening in a specific and more effective manner, with fewer complications and, possibly, with curative and preventive potential and not just purely symptomatic ones. The DEEs associated with the GRIN genes for the NMDA receptor are rare diseases that affect children, but the information resulting from this study will have a broader utility: they would in fact interest all patients suffering from drug-resistant epilepsy; furthermore, they could be of help for those who use the diet for other purposes (e.g. weight loss, muscular effectiveness), who often use it without calculating any possible damage to their health. The final objective is to promote knowledge of ketogenic therapy, to encourage good usage practices, but above all to research and develop valid substitutes, including new drugs or therapeutic strategies, in the interest of public health.
It is also important to underline that KD could have the potential not only to attenuate the intensity and number of epileptic seizures, which represent the most acute debilitating aspect in these syndromes, but could also induce beneficial effects on the normal process of neurodevelopment and on cognitive functions. The translational potential is high because this research involves the acquisition of important and still scarce knowledge relating to the molecular and physiological mechanisms underlying the effectiveness of the diet.

c. General aim and integration with mission of the Institute

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. Another objective of our projects is the search for new therapeutic targets and treatments for various neuropathologies and their comorbidities. Actually, our first line of research has great clinical relevance for the development of therapies for children, adolescents and adults affected not only by rare epileptic syndromes, but also by other forms of drug-resistant epilepsy and neurodevelopmental disorders, in which there is an imbalance between excitatory and inhibitory neuronal transmission. For this reason, our purposes fit very well with the mission of the Institute.

d. Specific objectives and strategies

1. 'Diet and Health Project: impact of the ketogenic diet in the treatment of drug-resistant epilepsy
GluN2A(N615S) mice (homozygous, heterozygous and wild type, male and females) will be fed KD or standard diet (SD) from the time of weaning [postnatal day 30 (P30)]. Both the KD and SD diets will be provided by a company specialized in the creation of special dietary regimes for experimental purposes for small rodents. The prepubertal age of initiation of KD treatment was chosen to: 1) mimic as much as possible the typical use of KD for the treatment of pediatric or adolescent epileptic patients; 2) align the age of murine brain development with that of human brain development considering that the main stages of brain neurodevelopment in the 4-week-old mouse are comparable to those of a human brain of a child aged 3-4 years and older and 3) avoid treatment during the pre-weaning period as the effects of the diet on maternal physiology and behavior could be confounded with the direct effects of the diet on the offspring. Mice will be subjected to KD treatment for 5 weeks (around P65). Mice belonging to each of the twelve experimental groups will be subjected to a battery of behavioral tests starting from the fourth week of the diet (P56), at the end of which (P65) they will be tested for AGS and subjected to a humanitarian endpoint. The duration of treatment has been established based on previous literature data.
Ex-vivo, we will analyze the expression of marker of neuronal plasticity and of neuroinflammation by immunohistochemistry on brain slices, in particular in encephalic regions with epileptogenic activity, identified by the alteration of c-fos expression. In collaboration with Prof Collino laboratory, the systemic glycemic 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 objective of the project was to screen several compounds with known and putative effects on MMP-9/ECM using an in vitro assay and human FXS fibroblasts (thanks to collaborators from Rome) and evaluate compounds with known effects on MMP-9 on Fmr1KO mice from molecular aspects (PNNs and their components) to synaptic, cognitive and behavioral deficits and, finally, assess efficient compounds found through the in vitro screening in Fmr1KO mice and in human FXS iPSCs-derived neurons (always thanks to collaborators).
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. The final aim is to find out future clinical candidates for FXS treatment.
3- 'Astrocyte NMDAR activity in cortical learning'
Based on our previous study (Hasan et al., 2013) and recently published research (Bertocchi et al., 2023; Carretero-Guillén et al., 2024), we would like to better investigate the role of astrocyte NMDARs in the

modulation of synaptic activity and learning and memory processes. Localized Ca2+ microdomain activity can be detected in astrocytes during information processing in the brain. However, the source and functional organization of these Ca2+ 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 have generated astrocyte-specific Grin1 gene knockout mice by using rAAVs for Cre recombinase-dependent gene deletion under the GFAP promoter (paper under revision) and, in collaboration with NICO's members, we will perform in vivo two-photon microscopy in these control and KO mice.
4) A novel mouse model of autism (paper under revision) will be further characterized behaviorally and by ex-vivo analysis.

e. Unique features of the project research

A significant feature of our project research is its high translational potential: the main objective of our projects is in fact to develop novel therapies in a preclinical setting focusing on conditions with unmet medical needs. Despite the loss of our PI at the end of 2023, making us the smallest group in the Institute, we are continuing to produce excellent science and publications and at a higher rate. This is possible thanks to our commitment and also the propensity to collaborate and share knowledge. In fact, the project makes use of the collaboration of a network of experts in both basic and clinical sciences, as well as the support of the association of parents of children affected by rare syndromes (https://www.gri-italia.it). This alliance gives us the determination and drive we need to continue to be strong and determined despite difficulties and defeats.

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2024

Laboratory name: **Neuroendocrinology**

1. LABORATORY DESCRIPTION – PERSONNEL:

Principal Investigator (Acting Group leader)
Gotti, Stefano, Associate Professor, PhD, 17/06/1971, 0116706610, stefano.gotti@unito.it

Personnel
Marraudino, Marilena, Assistant ProfessorRTD-B, PhD, 08/06/1988, 0116706632, marilena.marraudino@unito.it,
Control of reproduction, endocrine disruptors

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Eating and gaming disorders models

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Ricci, Elena, DNS Research fellow, Master Degree, 12/06/1998, 0116706632, elena.ricci@edu.unito.it,
endocrine disruptors

2. CURRENT GRANTS

Starting-end date	Project Title and ID	Beneficiary	Funding Program/Agency	Role of the unit	Overall AmountFunded	Managed by FCO/UNITO
2021-2024 (end 04.25)	Developmental, Reproductive and Metabolic effects of Endocrine Disruptors: the DReaM-ED study	Gotti, PI	PRIN 2020	PI of research unit	134696	UNITO
2024-2026	Fitoestrogeni e diete ipoproteiche in madri con Anoressia Nervosa, quali le alterazioni per il neurosviluppo della prole?	Marraudino	Fondazione CRT	Coordinator	25000	FCO

3. SCIENTIFIC ACTIVITIES IN 2024

Stefano Gotti, PI

Supervised PhD students:	Chiara Ballan Antonino Casile (with Prof. Cifani, University of Camerino)

Honors, prizes, awards:	
Outreach activities	
• International collaborations:	Cooperation with Prof. P. Collado and H. Pinos (UNED, Madrid, Spain) Cooperation with Prof. D. Grassi (Universidad Europea de Madrid, Madrid, Spain)
• Invited talks:	Role of stress, gonadal hormones, and diet in the Activity-Based-Anorexia Rat model, Congresso SIF (15/11/2024)
• Science communication:	Piattaforma Plastiche sostenibili SusPlas@Unito
• Editorial duties:	Journal Reviewer: Brain Research, Cell and Tissue Research, Physiology and Behavior, Neurological Science, Toxics, Chemosphere, Cells, Neurobiology of Disease, Molecular and Cellular Neuroscience, Journal of Neuroendocrinology, Behavioral Brain Research
• others	
Organizational activities and responsibilities at NICO:	First aid and fire safety officer Responsible for Cryostat Room Responsible for Perfusion Room Responsible for -80 Room
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	Organizer of the 12th Meeting Steroids and Nervous System, Torino, February 24-27, 2024 Organizer of the SNS Webinar (25/10/2024)
Technology transfer achievements (patents, etc.):	

Marilena Marraudino, Assistant Professor RTD-B

Supervised PhD students:	N.A.
Honors, prizes, awards:	Premialità RTD-B 2024 from Università degli studi di Torino
Outreach activities	
• International collaborations:	• Prof. Daniela Grassi, Neuroanatomy and Neuroendocrinology, Faculty of Basic Biomedical Science, Universidad Europea de Madrid, Madrid, Spain. • Prof. Paloma Collado, Neuro Psychoendocrinology, National Distance Education University (UNED), Department of Psychology, Madrid, Spain • Prof. Luis Miguel Garcia Segura, Neurodevelopment and Neuroinflammation, Cajal Institute, Neuroactive steroids, Madrid, Spain. • Dr. Matthieu Keller, Neuroendocrinology, INRA, Neuroendocrinologie des Interactions et Comportements Sexuels, Tours, France.
• Invited talks:	11/11/2024 - Invited by Dr. Silvia Diviccaro for a communication entitled 'Phytoestrogens as EDCs, when the term “natural” is not always synonymous with safety'. Course 'Health effects of endocrine disruptors', Dept. of

	Pharmacological and Biomolecular Sciences, University of Milan, Italy. 16/07/2024 - 5th Edition of World Congress on Endocrinology, Diabetes, and Metabolism (EDM-2024 Congress), Vienna, Austria, Jul 15 - 16, 2024, communication 'Low-dose perinatal treatment with Bisphenols (BPA or BPS) alters maternal care in dams and reproductive behavior in adult offspring mice'.
• Science communication:	U*NIGHT - La Notte Europea dei Ricercatori e delle Ricercatrici 2024, Caffe Scientifico (28/09/2024) - Come gli alimenti 'interferiscono' sul nostro cervello. Ricercatore in classe. Interferenze sul cervello. 07/03/2024. Prof. Annarita Deluca, Istituto di Istruzione Superiore Santorre di Santarosa. 26/05/2024. Prof. Ivan Enrico Repetto, IIS Primo Levi.
• Editorial duties:	• 2024 – Reviewer for Hormones and Behavior, Official Journal of the Society for Behavioral Neuroendocrinology. • since 2023 - Topical Advisory Panel Member of section "Molecular Endocrinology and Metabolism" in the International Journal of Molecular Sciences (IJMS) of Multidisciplinary Digital Publishing Institute (MDPI).
• others	2024 - Member of the educational committee,12th International Meeting Steroids and Nervous System (SNS) – February 2024, Turin, Italy.
Organizational activities and responsibilities at NICO:	Responsible for Cryostat Room Responsible for Perfusion Room
Speakers invited:	• Silvia Diviccaro, University of Milan • Silvia Giatti, University of Milan
Other organizational activities:	
Workshops, Schools or Conferences organized:	Local Organization of Satellite Symposium. Pharmacological modulation of steroidogenesis and steroid effects in the nervous system - SNS. Turin, Italy (24/02/2024). Organization of 1st SNS Webinar aimed to promoting young talented researchers (25/10/2024).
Technology transfer achievements (patents, etc.):	N.A.

4. Research activity in 2024

a. Summary

In 2024, we primarily focused our activities on anxiety-related behaviors. On one hand, we examined the effects of exposure to endocrine disruptors on various neural circuits and associated behaviors. On the other hand, we investigated the impact of gaming disorders on anxiety behavior.

b. Background and rationale

In the nervous system, many biological effects are mediated by steroid hormones: nuclear estrogen receptors (ERα and ERβ) and membrane receptors (GPER-1) are expressed in various brain regions during ontogeny. Estrogens may modulate neuronal differentiation, particularly by influencing cell migration, survival and death, and synaptic plasticity. Therefore, appropriate levels of gonadal hormones are essential for the normal development and sexual differentiation of the central nervous system (CNS) and reproductive behavior. Disruption of this developmental environment through exogenous estrogen treatment or gonadectomy during critical periods of pre- and/or post-natal development may induce irreversible changes in the organization of the central nervous system and behavioral alterations in many species. Our studies focus on understanding what happens when this delicate hormonal balance is disrupted by external factors. For this reason, we conducted experiments that simulated an environmental change to observe its possible influence on behavior and the related neural circuits.

One of our research lines investigates substances known as Endocrine Disrupting Chemicals (EDCs), which can alter the functions of the endocrine system and are closely linked to the development and operation of the nervous system. Various EDCs include xenoestrogens and xenoandrogens, which can significantly affect the development and function of gonadal hormone-dependent neural circuits and associated behaviors. The impact of EDCs varies based on several factors, including the method, duration, and amount of exposure. Developmental stages are generally much more vulnerable to signal disruption than adult stages, and the effects of fetal or neonatal exposure may differ drastically from those of adult exposure.

Another research focus of our lab involves a rat model of gaming disorder (GD). GD is classified as a mental disorder and exhibits different characteristics in males and females, with a higher prevalence in males. Further research is needed to better understand the sex differences in GD, and animal models could help clarify the neurological basis of this disorder. We are particularly interested in how these differences affect addictive behavior and brain activity during play, as well as any potential changes in various circuits involved in the reward system.

c. Objectives

Our main goal was to study EDCs, particularly their interactions withneural circuits and behavior. We analyzed the effect of perinatal exposure to different EDCs and focused our attention on neuroendocrine circuits controlling feeding and anxiety behavior.

d. Results

Perinatal exposure to Endocrine Disrupting Chemicals

Exposure to endocrine-disrupting chemicals (EDCs) is particularly hazardous during specific critical periods of life when organisms are more vulnerable to hormonal changes. EDCs can affect various aspects of life, including maternal behavior.

Bisphenols, synthetic organic compounds utilized in plastic production, represent a highly prevalent class of EDCs. These substances, whether individual chemicals or mixtures, can interfere with various aspects of hormone activity.

Exposure to BPs can lead to various effects, particularly serious during critical life stages. By concentrating on perinatal exposure to BPA and its common substitute, BPS, we studied the effects on anxiety-related behaviors and the serotonergic system, which is essential for regulating these behaviors, in adult mice.

We treated C57BL/6J mouse 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 the treatment until the offspring were weaned (**Bonaldo et al., 2024**). Adult offspring of both sexes underwent behavioral tests, including the elevated plus maze and open field tests.

We analyzed the serotonergic system in the dorsal (DR) and median (MnR) raphe nuclei using immunohistochemical techniques. Behavioral tests revealed changes in BPA- and BPS-treated mice,

indicating differing effects of bisphenol exposure on anxiety-related behavior: anxiolytic in males and anxiogenic in females.
The analysis of the serotonergic system revealed sex differences in the dorsal raphe (DR) only, with control females showing higher levels of serotonin immunoreactivity (5-HT-ir) compared to control males. BPA-treated males exhibited a significant increase in 5-HT-ir across all analyzed nuclei, while BPS-treated males demonstrated an increase solely in the ventral DR.
In females, both bisphenol-treated groups showed a significant increase in 5-HT-ir in the dorsal DR compared to controls, and BPA-treated females also showed a significant increase in the MnR. These findings provide evidence that exposure to BPA or BPS during early life phases affects anxiety and raphe serotonergic neurons in a sex-dependent manner.

Rat model of gaming disorder
In 2018, the International Classification of Diseases (ICD-11) classified Gaming Disorder (GD) as a mental disorder. GD primarily affects adolescents who, after developing an addiction, display psychopathological traits such as social anxiety, depression, social isolation, and attention deficit.
However, various studies conducted in humans to date reveal several limitations, such as a lack of demographic diversity, unequal representation of age, differences in the type of games, and the duration of follow-up. Furthermore, no specific animal models are currently available for GD.
In our study (**Casile et al., 2024**), we present a novel GD rat model to investigate specific aspects of the disorder. This model addresses the lack of an experimental model for GD.
Two-month-old Wistar Kyoto rats, both male and female, underwent five weeks of training on a novel touch-screen platform. After the training period, the rats were evaluated for (a) their engagement with the activity under various conditions, (b) hyperactivity during gameplay, and (c) retention of these conditions after a break in the game and interruption of rewards. Following sacrifice, immunohistochemistry techniques were employed to analyze the immunoreactivity of c-Fos (a marker of neuronal activity) in various neural regions.
After the training, the rats subjected to the GD protocol displayed GD-related traits (e.g., hyperactivity, lack of control), and the behavioral phenotype was consistently preserved over time. These characteristics were entirely absent in the control groups. Finally, the analysis of c-Fos immunoreactivity in the prelimbic cortex (PrL), orbitofrontal cortex (OFC), nucleus accumbens, amygdala, and bed nucleus of the stria terminalis (BNST) revealed significant differences in the GD groups compared to the controls, indicating changes in neural activity linked to the development of the GD phenotype.
The proposal for a new GD rat model could act as an innovative tool for examining the behavioral and neurobiological aspects of this disorder in both sexes, the potential impact of external factors on predisposition and susceptibility, and the development of new pharmacological therapies.

e. Advancement in the field

Recent findings confirm that endocrine-disrupting chemicals (EDCs) can significantly affect pups exposed during the perinatal stage of life. We observed distinct changes in both males and females across various brain regions, along with modifications in some related behaviors. More importantly, our research has also demonstrated that one of the primary substitutes for BPA, BPS, may have harmful effects that are even worse than those of BPA.

f. Publications

Bonaldo B, Casile A, Ostuni MT, Bettarelli M, Nasini S, Marraudino M, Panzica G, Gotti S. 2024Perinatal exposure to bisphenol A or S: Effects on anxiety-related behaviors and serotonergic system. Chemosphere. 2024 Feb;349:140827. doi: 10.1016/j.chemosphere.2023.140827.
Research article – Q1

Casile A, Marraudino M, Bonaldo B, Micioni Di Bonaventura MV, Nasini S, Cifani C, Gotti S. 2024 Novel rat model of gaming disorder: assessment of social reward and sex differences in behavior and c-Fos brain activity. Psychopharmacology (Berl). 2024 Apr 5. doi: 10.1007/s00213-024-06576-y.
Research article – Q1

Melcangi RC, Giatti S, Gotti S. 2024 Editorial- State of the art on steroids and the nervous system: In memory of Giancarlo. Front Neuroendocrinol. 2024 Apr;73:101135. doi: 10.1016/j.yfrne.2024.101135.
Editorial – Q1

Fratini A, Mancini L, Liguori MG, Gotti S, Marchetti E. 2024 Uncovering of transplanted connective tissue graft: Clinical and histological evaluation. Clin Adv Periodontics. 2024 Jan 19. doi: 10.1002/cap.10278.
Case Report – Q3

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

We will develop multiple lines of research:

1 - Effects of perinatal exposure to bisphenols (BPA or BPS, or a combination of both) on the survival of mouse pups, kisspeptin hypothalamic circuits, and sexual-related behaviors. The kisspeptin system plays a crucial role in regulating important aspects of reproductive functions, including the onset of puberty and the estrous cycle, as well as certain behaviors like mate preference and lordosis. Additionally, the kisspeptin system is known to be influenced by endocrine disruption, although few studies have directly investigated the effects of bisphenols.

2—Effects of Genistein (and gonadal receptor hormone antagonists) on neural circuits that control reproduction, metabolism, and other physiological parameters. Genistein, a phytoestrogen commonly found in soybeans, demonstrates estrogen-like activity. It acts as an endocrine disruptor and poses particular risks when administered during specific "critical" developmental periods, such as the postnatal age.

3 - Effects of drug treatment in a rodent model of Gaming Disorder. After developing and validating the gaming disorder model, we aim to test several drugs assessed for addiction treatment on the model to observe the effects.

b. Background and Significance

Steroid hormones play crucial roles in the development, growth, maturation, differentiation, and protection of the central and peripheral nervous systems. They are synthesized from cholesterol and produced in various organs, including the adrenal glands, gonads, and placenta. Additionally, the nervous system itself can metabolize or *de novo* synthesize active steroids (*neurosteroids*), which may regulate the activity and survival of nerve cells.
Steroid hormones produced by gonads are implicated in the development of sexually dimorphic circuits and functions and in the control of physiological activities such 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 deal with the study of sex differences at any level, the effect of stimulating different estrogen receptors, and 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 known as 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 even in very low concentrations, they can deeply influence the development and function of gonadal hormone-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 on various factors, including the method of exposure, duration, and amount of exposure. The developmental stages are typically far more vulnerable to signal disruption than the adult stages, and the consequences of fetal or neonatal exposure may be drastically different from those of adult exposure.

What is the primary issue with EDCs? These environmental contaminants exhibit endocrine activity in humans, as well as in wildlife and domestic animal species. Some "natural" EDCs, such as the plant phytoestrogens commonly found in food, may significantly influence the reproductive cycles of small rodents and can have either positive or negative effects on other animals, including humans.
More recently, the concept of metabolic disruptors - substances that can induce profound alterations in metabolism - was developed. Another important environmental effect is linked to parental behavior.
In fact, it has been demonstrated that a lack of maternal cure may induce permanent alterations in some behaviors in the pups when they are adults, as well as permanent changes in neuroendocrine circuits.
In many cases, the effects are different in males and females, which is probably due to the involvement of the gonadal hormones in this mechanism.

c. General aim and integration with mission of the Institute

Our primary objective is to explore how steroid hormones interact with and regulate neural circuits that control essential physiological functions (e.g., reproduction, food intake, metabolism), paying special attention to gender differences. This goal closely relates to the neuroendocrine foundations of certain neurodegenerative diseases, which exhibit significant sexual dimorphism. Approaches to treating these diseases must always acknowledge that some fundamental mechanisms may vary by sex and/or depend on steroids. Additionally, environmental factors seem to influence the development of these diseases. Therefore, endocrine-disrupting compounds (EDCs) that may interact with steroid hormone receptors are key candidates for this environmental impact. Consequently, we plan to continue our projects focused on studying the neuroendocrine system, neurodegenerative and psychiatric diseases, and their potential connection with environmental factors.

d. Specific objectives and strategies

We will direct our research towards two main topics:

- **EDCs and steroid hormones effects**

Bisphenols effects.
We aim to investigate the effects of bisphenols on the hypothalamic kisspeptin system and reproductive behaviors in mice. The kisspeptin system plays a vital role in regulating reproductive functions, including the onset of puberty and the estrous cycle. Additionally, it is susceptible to endocrine disruption.

We will compare the effects of perinatal exposure to bisphenol A (BPA) or bisphenol S (BPS) on the kisspeptin system and reproductive behaviors in mice. From mating until the offspring are weaned, C57BL/6 dams will receive an oral treatment of 4 µg/kg body weight per day of BPA, BPS, or a vehicle alone. We will monitor the development of the offspring until postnatal day 90, at which point we will analyze their reproductive behavior.
Based on previous findings, we can hypothesize thatBPA (and likely BPS) may delay the onset of puberty and disrupt the estrous cycle in females. Additionally, both bisphenols are probably to impact sexual behavior in males. Through immunohistochemical analysis of the treated groups, we aim to reveal some changes in the hypothalamic kisspeptin system.
These results will support the notion that perinatal exposure to low doses of bisphenols affects certain reproductive-relevant parameters, sexual behaviors, and kisspeptin hypothalamic nuclei in a sexually differentiated manner.

GEN and Estrogens effects in pups.
As an endocrine disruptor, GEN may present risks, particularly during developmental stages. It causes changes in anxiety behavior, fertility, and energy metabolism, in addition to modifying specific brain circuits. Since the serotonin (5-HT) system plays a crucial role in many of these behaviors, we hypothesize that some of GEN's behavioral effects may stem from disruptions in the development of the 5-HT system. We will assess the impact of early postnatal exposure to GEN at a dose of 50 mg/kg body weight, which mimics the exposure level of infants consuming soy-based formulas, on anxiety-related behaviors and 5-HT neuronal populations in the raphe nucleus.
Male and female CD1 mice will receive oral treatment with GEN or a vehicle during their first eight days of life. On postnatal day 60, one group will undergo anxiety behavior testing, while another group will be euthanized for immunohistochemical analysis. Behavioral testing is expected to reveal differences in anxiety levels between the control and treatment groups. Immunohistochemical analysis of the raphe nuclei is expected to show changes in 5-HT neuronal counts in treated animals.
If confirmed, these findings will suggest that regulating anxiety-related behaviors and the 5-HT system are critical targets of early phytoestrogen exposure at levels comparable to those found in soy-based infant formulas.

Many factors, including gonadal steroids, influence and maintain the hypothalamic systems that regulate metabolism and reproduction during crucial developmental periods. Estradiol (E_2) seems to play an essential role in organizing these circuits. E_2 operates through three distinct receptors: two nuclear, ERα and ERβ, and one membrane receptor, GPR30.
As previously mentioned, the 5-HT system plays a crucial role in regulating anxiety-like behaviors and is sensitive to estrogen. Our goal is to investigate which estrogen receptor exerts its organizational effect on anxiety behavior, thereby influencing the distribution of 5HT+ neurons in the dorsal Raphe nucleus (DRN) and the median Raphe nucleus (MRN).
During the sensitive postnatal hormonal window, male and female CD1 mice will receive subcutaneous injections from postnatal day 5 to 12. They will be treated with E_2 alone or in combination with various selective estrogen receptor antagonists (MPP, PHTPP, and G15, which inhibit ERα, ERβ, and GPR30, respectively). In adulthood, the mice will participate in various behavioral tests to evaluate their anxiety levels and undergo immunohistochemical analysis of the 5-HT system in the Raphe Nucleus.

- **Translational studies**

Rat model of gaming disorder (GD)
Gaming Disorder (GD) is a mental health condition that primarily affects adolescents. Extended use of video games results in various behavioral changes, including loss of control over gaming, which is often accompanied by increased compulsiveness and hyperactivity.
These impairments are linked to changes in emotional states and the emergence of depressive and anxiety symptoms. Research in humans has shown alterations in the functioning of specific brain areas that regulate motivational states and reward circuits. Among the possible causes of these

changes is the altered activity of orexin (ORX), dopamine (DA), and serotonin (5-HT) positive neurons.
Recently, our laboratory developed a rat model that mimics some symptoms present in patients with GD. Building on this model, we aim to evaluate changes in these systems in rats exposed to the GD protocol using immunohistochemistry and high-performance liquid chromatography.
We expected to find some differences in the number of ORX+, DA+, and 5-HT+ cells between the groups, as well as some peripheral changes (tryptophan signaling, inflammatory metabolites) in their plasma levels.
Our results will support the hypothesis that impairment in these neurotransmitters plays a crucial role in GD and provide a foundation for further understanding some of the cerebral and behavioral changes observed in GD patients.

e. Unique features of the project research

Our research unit has always been interested in the interactions between steroid hormones and nervous tissue, using behavior as the main physiological endpoint. Numerous steroid hormone receptors are present in several brain areas, and it is known that steroid hormones play a role in neuronal and glial differentiation, survival, and protection. Therefore, we believe that gaining a better understanding of the relationships between steroid hormones and the nervous system is crucial. This interaction may partly explain gender differences in both physiological and pathological conditions.
Additionally, in the last two decades, the problem of how the environment can interact with human and animal physiology to induce pathologies has become an important topic for the biomedical sciences.
It is not surprising that many synthetic substances can interact with hormone receptors, potentially leading to endocrine imbalances and diseases. For many years, the neuroendocrine effects were underestimated, and nervous tissue was not a primary focus of research. Moreover, it was not considered a significant endpoint to include in the development of toxicological tests for regulating the use of EDCs.
Our research seeks to highlight the potential dangers posed by EDCs, especially regarding the central nervous system during development.
In summary, our research can enhance our understanding of gender differences in healthy brains, various neural pathologies, and the intricate interactions among neural circuits, behavior, and environmental contaminants.

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2024

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)

Position: Full Professor

PhD in Physiology, Master's degree in Biological Sciences, University of Turin

Birthdate: 25/02/1977

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Personnel

Ronchi Giulia

Position: Associate Professor

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

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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

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Email: luisa.muratori@unito.it

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

El Soury Marwa

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

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Role & expertise: Biomolecular analysis of peripheral nerve regeneration

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

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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

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Role & expertise: Biomolecular analysis of peripheral nerve regeneration; development of gold nanoparticles for *in vitro* and *in vivo* siRNA delivery.

Metafune Miriam

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

Degree: Master Degree in Molecular Biology, University of Parma

Birthdate: 18/12/1994

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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

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

Birthdate: 28/02/1998

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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: PhD student, PhD Program in Experimental Medicine and Therapy (from 01/02/2024)

Degree: Master degree in Medical Biotechnology, University of Turin

Birthdate:16/09/1997

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Email: francesca.bertone@unito.it

Role & expertise: *In vitro* models for nervous system regeneration, biochemical analysis of nervous tissue and behavioural tests on murine models.

Rando Simona

Position: PhD student, PhD Program in Experimental Medicine and Therapy (from 01/11/2024)

Degree: Master degree in Molecular Biotechnology, University of Turin

Birthdate: 02/03/1999

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Email: simona.rando@unito.it

Role & expertise: *In vitro* models of hiPSC for studying diabetic neuropathy

2. CURRENT GRANTS

Starting-end date	Project Title and ID	Beneficiary	Funding Program/Agency	Role of the unit	Overall AmountFunded	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 Unit	59.238	UNITO
10/2023 - 09/2025	Gut and NeuroMuscular system: investigating the impact of microbiota on nerve regeneration and muscle reinnervation after peripheral nerve injury (Gut-NeuroMuscle) 20227YB93W	Giulia Ronchi	PRIN-MUR	Coordinator	106.345	UNITO
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 Unit	107.130	UNITO

3. SCIENTIFIC ACTIVITIES IN 2024

Stefania Raimondo, (PI)

Supervised PhD students:	Federica Zen (PhD) Miriam Metafune (PhD) Simona Rando (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 LuísBombeiro -Kyushu Institute of Technology, Fukuoka, Japan, prof. Yuki Shirosaki
• Invited talks:	
• Science communication:	16/02/2024, LIVE con TrendSanità: "Nuovi orizzonti nella rigenerazione dei nervi: il futuro della ricerca"
• Editorial duties:	- Editorial Board Member of Frontiers in Neuroanatomy - Editorial Board Member of Biomedicines
• others	- Referee for grant agencies: FWO (Fonds voorWetenschappelijkOnderzoek - Vlaanderen) - President 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:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	Member of the Organizing Committee of the 7th International Symposium on Peripheral Nerve Regeneration (7nd ISPNR, 7-9 May 2024, Milano)
Technology transfer achievements (patents, etc.):	

Giulia Ronchi, Associate Professor

Supervised PhD students:	Riccardo Aucello (PhD) Davide Pellegrino (PhD) Francesca Bertone (PhD)
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	University of Hannover, Germany, prof Kirsten Haastert-Talini Faculdade de Medicina da Universidade de Lisboa, Dr. AndrèBombeiro University of Porto, Portugal, prof. Ana Colette Maurício and Prof. Rui DamásioAlvites
• Invited talks:	13th Workshop of Advanced Microscopy Techniques for Biomedical Applications, 05 June 2024 – "Transmission electron microscopy as a powerful tool for studying the peripheral nervous system"
• Science communication:	16/02/2024, LIVE con TrendSanità: "Nuovi orizzonti nella rigenerazione dei nervi: il futuro della ricerca" July 2024: Interview for TGRegione Piemonte 28/09/2024, Notte Europea dei Ricercatori – UNIGHT2024-Caffè scientifici: "Rigenerazione dei nervi: a che punto è la ricerca?"
• Editorial duties:	Youth Editorial Board Member di Neural Regeneration

	Research (NRR) Referee for Journal of Clinical Investigation, Antioxidants, International Journal of Molecular Sciences, Heliyoin Referee for the Book proposal - Peripheral Nerve Regeneration (Elsevier)
• others	
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	Member of the Organizing Committee of the 7th International Symposium on Peripheral Nerve Regeneration (7nd ISPNR, 7-9 May 2024, Milano)
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 with Stefania Raimondo)
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	University of Zaragoza, Spain, prof Jesus M. de la Fuente University of Granada, Spain, prof Victor Sebastian Carriel University of Hannover, Germany, prof Kirsten Haastert-Talini
• Invited talks:	
• Science communication:	16/02/2024, LIVE con TrendSanità: “Nuovi orizzonti nella rigenerazione dei nervi: il futuro della ricerca” 28/09/2024, Notte Europea dei Ricercatori – UNIGHT2024-Caffè scientifici: “Rigenerazione dei nervi: a che punto è la ricerca?”.
• Editorial duties:	Editorial Board Member of Brain Sciences Associate Editor for Cellular Neuropathology (section Frontiers in Cellular Neuroscience).
• others	
Organizational activities and responsibilities at NICO:	
Speakers invited:	Prof. Jesus M. de la Fuente, Institute of Nanotechnology and Materials of Aragón,CSIC/ University of Zaragoza, Spain, on “Designing Biofunctional Nanoparticles”, 14/03/2024-Seminar Prof. Bilal E. Kerman, Keck School of Medicine of USC - University of Southern California, USA, on “Exploring APOE4's Role in Alzheimer's Disease Using Stem Cells”, 22/11/2024-Webinar
Other organizational activities:	
Workshops, Schools or Conferences organized:	Member of the scientific committee of the 7th International Symposium on Peripheral Nerve Regeneration (ISPNR, 2024 Milan, Italy, 7-9 May 2024).
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:

	18/04/2018, expiry: 11/11/2035. Inventors: Stefano Geuna, Abraham Shahar, OfraZiv-Polat, Giovanna Gambarotta, Federica Fregnan.

Federica Fregnan, Research technician

Supervised PhD students:	Miriam Metafune (PhD)
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	- Kyushu Institute of Technology, Fukuoka, Japan, prof. Yuki Shirosaki - University of Hannover, Germany, prof Kirsten Haastert-Talini
• Invited talks:	The 7th Seminar for Young Researchers, February at Kyushu Institute of Technology, Kitakyushu, Japan, February,14th 2024. Communication entitled: "*In Vitro* and *In Vivo* Study of Autonomic and Somatic Nerve Regeneration Through Engineered Biomaterials".
• Science communication:	28/09/2024, Notte Europea dei Ricercatori – UNIGHT2024-Caffè scientifici: "Rigenerazione dei nervi: a che punto è la ricerca?".
• 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	Reviewer for Scientific International Journal (Frontiers, Acta Biomaterialia, BioMed Research International, MDPI)
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	Member of the scientific committee of the 7th International Symposium on Peripheral Nerve Regeneration (ISPNR, 2024 Milan, Italy, 7-9 May 2024).
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: 18/04/2018, expiry: 11/11/2035. Inventors: Stefano Geuna, Abraham Shahar, OfraZiv-Polat, Giovanna Gambarotta, Federica Fregnan.

Luisa Muratori, Research Assistant

Supervised PhD students:	Miriam Metafune (PhD)
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	University of Zaragoza, Spain, prof Jesus Ciriza
• Invited talks:	
• Science communication:	

• Editorial duties:	
• others	Oralpresentationat meeting: 7th International Symposium on Peripheral Nerve Regeneration ISPNR, 7-9 Maggio 2024, Milano. Abstract title: "Repair of a rat median nerve injury using a novel decellularized porcine nerve graft: a preliminary *in vivo* study".
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	Member of the organizing committee of 7th International Symposium on Peripheral Nerve Regeneration (ISPNR, 2024 Milan, Italy, 7-9 May 2024).
Technology transfer achievements (patents, etc.):	

4. Research activity in 2024

a. Summary

Our research is primarily focused on understanding the complex biomolecular and biological processes involved in peripheral nerve repair and regeneration. We aim to uncover new strategies for repairing severe somatic and autonomic nerve injuries and enhancing overall peripheral nerve regeneration. Additionally, we have recently expanded our research to explore the influence of microbiota on the development of the peripheral nervous system. These investigations are being conducted through both *in vitro* and *in vivo* models to provide comprehensive insights into these processes.

b. Background and rationale

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 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

The group's objectives were to elucidate the biomolecular and biological processes underlying nerve regeneration and to explore strategies for enhancing functional recovery following 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 immediate or 3 months-delayed nerve gap repair using different conduits; ii) studying the effect of gut microbiota on the peripheral nervous system development; iii) Studying biomaterials as possible nerve repair device; iv) evaluating allograft efficacy to repair nerve lesions with substance loss; v) studying new strategies to improve outcome after periprostatic nerve bundles injury; vi) developing gold-nanoparticle conjugated siRNAs to study the role of different factors during nerve regeneration; vii) development of an *in vitro* model using human induced pluripotent stem cells (hiPSC)

d. Results

i) Studying biological events during peripheral nerve regeneration.

Conduits used to bridge nerve gaps are effective like autograft (the “gold standard” technique) when gaps are short and the repair is immediate. Understanding nerve regeneration within short gaps could help improve their efficacy for longer gaps. Endothelial cells form a capillary network used by Schwann cells to colonize conduits before axon regrowth. As “muscle-in-vein” is a 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.

Additionally, we demonstrated that Schwann cell-specific ablation of Beclin 1, a key component of the class III phosphatidylinositol 3-kinase complex, impairs myelination, critical for peripheral nerve function.

ii) Effect of gut microbiota on the peripheral nervous system development

The analysis of the effect of the microbiota metabolite short-chain fatty acids (SCFA) on Schwann cell activity *in vitro* showed that SCFA exert an antioxidant effect on primary Schwann cells. In addition, analysis of nerves from 'ex-GF' mice (i.e. born and raised in a germ-free environment until weaning and then housed with specific pathogen-free (SPF) mice for microbiota re-colonisation), showed that the nerve fibres are (again) hypermyelinated. Further microbiome and metabolomics analyses are ongoing.

iii) Studying biomaterials as possible nerve repair device;

Innovative materials have been evaluated for their ability to promote nerve regeneration in somatic and autonomic injuries:

a) Glucidex®-derived membranes, made from maltodextrin, were tested *in vitro* with nervous cells. The dissolution products supported Schwann cell proliferation, with organized actin cytoskeleton and lamellipodia, indicating cell migration crucial for glial support in nerve regeneration. No link to cell death was found when analyzing apoptosis-related proteins (Bax and Bcl-2).

b) Zein-derived membranes, made from a hydrophobic protein in corn, were tested for biocompatibility and biodegradability. *In vitro* studies on a glial cell line showed excellent biocompatibility, supporting direct culture contact and potential for drug delivery applications.

c) Decellularized extracellular matrix (dECM) hydrogel from human cadaver skin was tested to assess its ability to promote Schwann cell proliferation, migration, and neurite outgrowth in both sensory and motor neuronl cell lines and primary sensory neuron cultures.

iv) Evaluating allograft efficacy to repair nerve lesions with substance loss;

In vivo experiments on rats allowed to evaluate the efficacy of decellularized porcine nerves as a xenograft model to repair a nerve injury. The post recovery time was extended to 12 weeks after

injury/repair to evaluate the functional recovery and to perform stereological assessment of regenerated fibers.

v) Studying new 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.
Nanopatterned and flat chitosan membranes were applied *in vivo* to repair cavernous nerve injury on adult rats. The post recovery time was extended to 120 days to evaluate the ability of the devices to allow autonomic axonal regeneration and functional recovery. Furthermore, deep sequencing analysis is ongoing to evaluate differentially expressed genes in regenerated nerves and target organs 120 days after repair.

vi) Development of gold-nanoparticle conjugated siRNAs
In collaboration with the University of Zaragoza, siRNAs targeting different isoforms of the same factor (Neuregulin1) were developed, tested *in vitro* and conjugated with gold-nanoparticles for *in vivo* tests that will be carried out next year.

vii) Development of an *in vitro* model using human induced pluripotent stem cells (hiPSC)
To develop a "human" peripheral nerve *in vitro* model, human induced pluripotent stem cells (hiPSC) were grown and stimulated to differentiate towards a motor neuron phenotype.
Fibers, with random or aligned orientations, were generated using electrospinning technology, employing a blend of polycaprolactone and polyaniline, doped with camphor-10-sulfonic acid, followed by atmospheric plasma treatment. Proliferating or differentiated hiPSC, and primary cultures of Schwann cells or dorsal root ganglia, were successfully cultured on alignedfibers.

e. Advancement in the field

The main advancements reached with our research activities during 2024 can be summarized as follow: i) vascularization plays a key role during nerve regeneration; it might be impaired when repair is delayed, but improved by conduit enrichment with muscle fibres; ii) the gut microbiota has an effect on the development and activity of the peripheral nervous system; iii) new promising biomaterials were efficiently tested *in vitro* for nervous cell biomimeticity; iv) *in vivo* implantation (for the first time) of a decellularized porcine nerve to repair a rat median nerve injury supports nerve regeneration; v) we demonstrated the regeneration of the autonomic nervous system after damage and repair with chitosan membranes, thus mimicking in rats the iatrogenic injury of the neurovascular bundles after radical prostatectomy in humans; vi) siRNAs were developed, *in vitro* tested and conjugated with gold nanoparticles for efficient and controlled *in vivo* delivery; vii) hiPSC were grown and stimulated to differentiate towards a motor neuron phenotype and cultured on aligned fibers.

f. Publications

1. Cescon M, **Gambarotta G**, Calabrò S, Cicconetti C, Anselmi F, Kankowski S, Lang L, Basic M, Bleich A, Bolsega S, Steglich M, Oliviero S, **Raimondo S**, Bizzotto D, Haastert-Talini K, **Ronchi G**. Gut microbiota depletion delays somatic peripheral nerve development and impairs neuromuscular junction maturation. Gut Microbes. 2024 Jan-Dec;16(1):2363015.
Research article Q1

2. Lembo D, Abate Daga F, Calì C, Garbossa D, Manfredi M, Odetto L, Ostacoli L, Paccotti P, **Raimondo S**, Reimondo G, Sciascia S. Early introduction of simulation in the medical curriculum: the MedInTo perspective. Front Med (Lausanne). 2024 Jan 4;10:1280592.
Research article Q1

3. Altun G, Önger ME, **Geuna S**, Elhaj AE, **Raimondo S**, Deniz ÖG, Kaplan S. The amelioration effects of ankaferd blood stopper, platelet gel, and Momordica charantia on peripheral nerve injury in the rats: a stereological and ultrastructural study. Histochem Cell Biol. 2024 Nov 18;163(1):5.
   Research article Q2

4. Gambarotto L, Russo L, Bresolin S, Persano L, D'Amore R, **Ronchi G**, **Zen F**, **Muratori L**, Cani A, Negro S, Megighian A, Calabrò S, Braghetta P, Bizzotto D, Cescon M. Schwann Cell-Specific Ablation of Beclin 1 Impairs Myelination and Leads to Motor and Sensory Neuropathy in Mice. Adv Sci (Weinh). 2024 Dec 16:e2308965.
   Research article Q1

5. **Muratori L**, Crosio A, **Ronchi G**, Molinaro D, Tos P, Lovati AB**, Raimondo S**. Exploring an innovative decellularization protocol for porcine nerve grafts: a translational approach to peripheral nerve repair. Front Neuroanat. 2024 Mar 19;18:1380520.
   Research article Q1

6. Damiati LA, Durand-Herrera D, **El Soury M**. Editorial: The future of modern medicine: cells, scaffolds, and biofactors. Front Med (Lausanne). 2024 Jun 27;11:1434760.
   Research article Q1

7. Damiati LA, **El Soury M**. Bone-nerve crosstalk: a new state for neuralizing bone tissue engineering-A mini review. Front Med (Lausanne). 2024 Apr 16;11:1386683.
   Research article Q1

8. Sousa P, Lopes B, Sousa AC, Coelho A, de Sousa Moreira A, Rêma A, Gonçalves-Maia M, Amorim I, Alvites R, Alves N, **Geuna S**, Maurício AC. Isolation, Expansion, and Characterization of Rat Hair Follicle Stem Cells and Their Secretome: Insights into Wound Healing Potential. Biomedicines. 2024 Dec 15;12(12):2854.
   Research article Q2

9. Bourqqia-Ramzi M, Mansilla-Guardiola J, Muñoz-Rodriguez D, Quarta E, Lombardo-Hernandez J, Murciano-Cespedosa A, Conejero-Meca FJ, Mateos González Á, **Geuna S**, Garcia-Esteban MT, Herrera-Rincon C. From the Microbiome to the Electrome: Implications for the Microbiota-Gut-Brain Axis. Int J Mol Sci. 2024 Jun 5;25(11):6233.
   Research article Q1

10. Lysak A, Farnebo S, **Geuna S**, Dahlin LB. Muscle preservation in proximal nerve injuries: a current update. J Hand Surg Eur Vol. 2024 Jun;49(6):773-782.
    Research article Q2

11. Lopes B, Coelho A, Alvites R, Sousa AC, Sousa P, Moreira A, Atayde L, Salgado A, **Geuna S**, Maurício AC. Animal models in peripheral nerve transection studies: a systematic review on study design and outcomes assessment. Regen Med. 2024 Apr;19(4):189-203.
    Research article Q3

5. Future directions and objectives for next years

a. Summary

The research group aims to explore innovative therapies to improve patient outcomes following somatic and autonomic peripheral nerve injuries. Both cutting-edge and established *in vitro* and *in vivo* experimental models will be utilized to achieve this goal. Specifically, glial and neuronal *in vitro* models will be employed to evaluate innovative biomaterials for potential use as nerve repair prostheses. Ongoing studies will focus

on GLUCIDEX®-derived membranes, Zein-derived membranes, dECM hydrogel, and functionalized chitosan membranes to deepen our understanding of their potential for *in vivo* applications.
Additionally, a "nerve-on-a-chip" system will be developed as a novel *in vitro* model for nerve studies, using human-induced pluripotent stem cells (hiPSC) cultured on a 3D scaffold. The regeneration of nerves within various artificial and biological conduits will be further examined, with particular attention to decellularized porcine nerve grafts, empty and filled conduits, and membranes made from different biomaterials. These studies aim to assess the ability of these materials to support nerve regeneration and the role of different cell populations and factors in the process, with the goal of improving outcomes for larger nerve gap repairs.Molecular mechanisms will be analysed for a better understanding of the nerve regeneration process. In particular, the roles of Neuregulin1, RGS16 and Beclin1 will be investigated.
Finally, the research group plans to expand its focus on the innovative and emerging topic of microbiota alterations and their potential involvement in peripheral nervous system development and nerve regeneration after traumatic injury. This research could provide new insights into novel therapeutic strategies, such as microbiota modulation, to potentially enhance nerve regeneration and improve outcomes for patients suffering from peripheral nerve disorders.

b. Background and Significance

Studying the relationship between microbiota and peripheral nerve structure and function.
We have recently demonstrated a regulatory impact of the gut microbiota on proper development of the somatic peripheral nervous system and its functional connection to skeletal muscles.

Over-expression of RGS16 after acute nerve injury
Open-access single-cell RNA sequencing datasets were analyzed, focusing on mice intact sciatic nerves and distal stumps at 3- and 9-days post-injury. Rgs16 emerged as the Regulators of G Protein Signaling (RGS) member most highly expressed by Schwann cells after injury, suggesting its involvement in nerve degeneration and regeneration.

Neuregulin1 α and β
After nerve injury and repair different Neuregulin1 (NRG1) isoforms - type α and type β - are expressed. It is known that NRG1 plays a key role in Schwann cell dedifferentiation and proliferation and that type α is mainly released by nerve fibroblasts, while β is released by both fibroblasts and Schwann cells. To further investigate their role *in vivo*, short interfering RNA (siRNA) targeting NRG1 α or β were developed, tested and functionalized (in collaboration with Zaragoza University) with gold nanoparticles to promote internalization.

Delayed repair after nerve injury
Conduits used to bridge nerve gaps are effective like autografts (the "gold standard" technique) when the repair is immediate. Messenger RNAs transcribed within a conduit repaired after injury with a 3 months delay were sequenced and compared with those expressed after immediate nerve repair. Interestingly, several signaling pathways show a different behavior.

Role of Beclin 1 into myelin maintenance and peripheral nerve regeneration
A better understanding of the molecular mechanisms behind myelin maintenance and Schwann cell-axon interactions is crucial for developing strategies to modulate Schwann cells and sustain their repair phenotype in clinical settings, ultimately enhancing and accelerating nerve regeneration in humans. We have shown that constitutive ablation of Beclin-1 in Schwann cells leads to severe demyelination.

Studying biomaterials as possible nerve repair device
Innovative materials have been assessed *in vitro* for their ability to promote nerve regeneration, a) Glucidex®- derived membranes; b) Zein- derived membranes; c) decellularized extracellular matrix (dECM) hydrogel. Preliminary experiments demonstrated their potential use for repairing somatic and autonomic lesions is promising.

Evaluating different techniques to repair nerve lesions with substance loss
Decellularized porcine nerves have demonstrated, 4 weeks after surgery, to support *in vivo*, on a rat model of median nerve lesion, the nerve fiber regeneration. For this reason, the post recovery time was extended to 12 weeks. Functional analysis is ongoing and morphological and stereological assessment of regenerated fibers will be done.

Studying new strategies to improve outcome after periprostatic nerve bundles injury
Erectile dysfunction after radical prostatectomy for cancer removal is still an important problem that affects patients' 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.Flat and nanostructured membranes with two different topographies, grating arrangement and zig-zag pattern were applied *in vivo* to repair cavernous nerve injury on adult male rats and their success in promoting nerve fiber regeneration 60 days after the surgical procedure was demonstrated.

Development of an *in vitro* model using human induced pluripotent stem cells (hiPSC)
Developing a "nerve-on-a-chip" system for peripheral nerve studies using human cells 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.

c. General aim and integration with mission of the Institute

The group's main goal is to explore innovative strategies to enhance functional recovery following traumatic and iatrogenic nerve injuries. Nerve damage is a leading cause of neuronal disability, profoundly affecting patients' quality of life, including their psychosocial and relational well-being. Advancing treatment for these patients requires a collaborative approach that integrates both CNS and PNS researchers, aligned with the mission of NICO.

d. Specific objectives and strategies

Studying the relationship between microbiota and peripheral nerve regeneration
Future research will be focused on elucidating the specific roles of the gut microbiota composition and metabolites, as well as the signaling pathways through which they may influence nerve degeneration and regeneration.

Studying the dynamic regulation of RGS16 expression in acute and chronic nerve injury
Rgs16 expression will be assessed at mRNA level at different time points in the median nerve of adult rats under regenerating conditions following mild (crush) or more severe (end-to-end) traumatic injury, and under degenerating conditions, in both acute and chronic nerve injury, to investigate its possible role in nerve degeneration and regeneration.

Role of Neuregulin1 α and β *in vivo*
siRNA targeting NRG1 α or β functionalized with gold nanoparticles will be used *in vivo* to study the role of the different NRG1 isoforms in nerve degeneration and regeneration. Indeed, the role of the different isoforms needs further investigation to understand which isoform is more useful to promote nerve regeneration through *in vivo* controlled delivery.

Morphological analysis of nerve regeneration within a conduit after delayed nerve repair
To investigate the effect of delayed repair on vascularization, Schwann cell migration, and nerve regeneration, morphological, morphometrical and biomolecular analyses will be carried out at 14 and 21 days after immediate and 3 month-delayed repair of a nerve gap with a chitosan conduit.

Role of Beclin1 into myelin maintenance and peripheral nerve regeneration
Currently, we are investigating the *in vivo* impact of Beclin1-regulated processes on myelin maintenance, nerve regeneration, and skeletal muscle innervation using an inducible Schwann cell-specific Beclin1 knockout mouse model, where the gene is deleted in response to tamoxifen.

Studying biomaterials as possible nerve repair device
The analysis of the efficacy of the innovative materials (Glucidex®- derived membranes; Zein- derived membranes; dECM hydrogel) preliminary tested for their ability to promote nerve regeneration will continue with further *in vitro* experiments and *in vivo* for the most promising between them.

Evaluating different techniques to repair nerve lesions with substance loss
The morphological and morphometrical analysis of regenerated nerve fibers 12 weeks after rat median nerve injury/repair with decellularized porcine nerves will be performed. Moreover, short term analysis (7 days) will be done in order to evaluate inflammatory processes.

Studying new strategies to improve outcome after periprostatic nerve bundles injury
Nanostructured chitosan membranes previously used to repair prostatic nerves in rats, will be functionalized 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.

Development of an *in vitro* model using human induced pluripotent stem cells (hiPSC)
Future aims will be to set up an *in vitro* model of cell cultures of Schwann cells, motor neurons and/or sensitive neurons derived from human induced pluripotent stem cells (hiPSC) and to develop a bioengineered "nerve-on-a-chip" by co-culturing human Schwann cells and neurons on a 3D scaffold in order to study the effects of different treatments (with drugs, gut bacteria metabolites, …) on cell cultures and on the bioengineered peripheral nerve.

e. Unique features of the project research

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 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.

f. Methodology

The most innovative aspects of our research will be:
1. The use of hiPSC as *in vitro* model for the study of peripheral nerve
2. The extensive use of computational biology/bioinformatics techniques

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2024

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

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Role & expertise: Pathogenesis of Alzheimer's disease and molecular biology

CURRENT GRANTS

Starting-end date	Project Title and ID	Beneficiary	Funding Program/Agency	Role of the unit	Overall AmountFunded	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

2. SCIENTIFIC ACTIVITIES IN 2024

Elena Tamagno, Role (PI)

Supervised PhD students:	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 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:	4th International Conference on COGNITIVE AND BEHAVIORAL NEUROSCIENCES.11-13 September 2024 Lisbon; 42° Congresso Nazionale SIF Società Italiana di Farmacologia 13-16 November 2024, Sorrento
• Science communication:	Notte Europea delle Ricercatrici e dei Ricercatori UNIGHT, Musei Reali, Torino 28 settembre; Festival della Scienza di Settimo Torinese, Settimo Torinese 12 ottobre
• Editorial duties:	Associate Editor in Molecular Diagnostics and Therapeutics of Frontiers in Molecular Biosciences
• others	
Organizational activities and responsibilities at NICO:	Responsible for the Cell Culture room (floor 0) Responsible for the dissection room (floor -1) Responsible for Western Blot Lab (floor 0) Responsible for Western Blot /Bacteria Lab (floor -1)
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Giulia Morello PhD student

Activities:	Ricercatori alla Spina - Brain Edition - 23 March 2023 - Divulgazione Scientifica International Brain Research Organisation (IBRO) 9-14 September 2023, Granada, Spain Poster Presentation: miRNA-218 as a putative biomarker for early diagnosis in Alzheimer's disease in female patients" Tissue Networks - from Imaging to Quantification and Modeling Summerschool 28 September 2023, Wurzburg, Germany Oral Presentation: Fasting: from evolutionary advantage to a new strategy against neurodegeneration FENS forum 2024 – 25-29 June 2024, Wien, Austria Poster Presentation: Protective effect of growth hormone-releasing hormone (GHRH) agonist MR-409 in Alzheimer's disease

	Riunione Scientifica della Scuola Dianzani, Genoa, 18 settembre Oral Presentation: Sleep fragmentation accelerates Alzheimer's disease pathology in 5xFAD mouse model and affects memory consolidation in young wild-type mice through glymphatic system alteration

4. Research activity in 2024

a. Summary

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.

b. Background and rationale

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).

c. Objectives

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.

d. Results

The two types of EVs were administered to male 3-month-old mice, following an intranasal route to bypass the blood brain barrier and reach target regions in the brain. In 5xFAD mice, our data seem to suggest an effective role of both types of vesicles in reducing astrogliosis, not only in the more anterior regions of the brain, as expected, but also in the hippocampus and cortical areas, which play a crucial role in Alzheimer's disease. In addition, a consistent decrease in the density of plaque deposits can be described in the very same regions: this seems to be especially true for OGD MSC-EVs, but whether these vesicles can have different effects of different targets and regions is still to elucidate. Unfortunately, 5xFAD animals show no improvement from a behavioural point of view. This could be importantly correlated with the phenotype severity that characterises this mouse strain, with a massive plaque deposition that starts already at 2-months of age and that could easily lead to non-reversible changes in behaviour. Nevertheless, wild-type animals subjected to the very same treatments showed lower anxiety levels as well as improved discrimination of objects during the test, thus suggesting that EVs can effectively reach the brain, target brain regions involved in memory and improve the performance of the animals in the maze. Preliminary results on hTau animals seem to confirm these data, with discrimination index scores which are higher when compared with hTau animals treated with PBS as control.

e. Advancement in the field

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.

f. Publications

5. Future directions and objectives for next years

a. Summary

According to data from the World Health Organization (WHO), Alzheimer's disease (AD) is most common form of dementia and is a condition that afflicts 50 millionpeople worldwide, with about 10 million new cases each year. To date, there are about 1,200,000 people with dementia in Italy; in the Piedmont Region alone, a conservative estimate, updated as of January 1, 2021, estimates that there are about90,000 sufferers over 65. The primary neuropathological

events in Alzheimer's disease and all other forms of dementia involve the aberrant formation of pathological protein species. Although science has provided the necessary tools to unravel the mechanisms underlying the synthesis, modification, and precipitation of these proteins, the pathogenesis of brain tissue damage begins many years before the onset of neurological signs. Moreover, several Authors demonstrate the existence of a gender prevalence in the disease, as women are more susceptible to develop AD than men. Therefore, the complexity of this condition suggests a broad, integrated care with ad hoc, flexible and personalized interventions. In the absence of effective therapy, the search for predictive plasma biomarkers, allowing early identification of the disease, seems to be the only approach that can pave the way for useful therapies to slow the onset and progression of the condition.

b. Background and Significance

Alzheimer's disease is classified into two forms: the sporadic form, which accounts for most cases and has a late onset, and the familial form, a rare and early-onset form. The later form is due to genetic mutations, such as those in the APP, PS1, PS2 genes, while the sporadic form is a multifactorial syndrome. At the neuropathological level, AD is characterized by intracellular neurofibrillary tangles, in which the main constituent is hyperphosphorylated tau protein; extracellular β- amyloid aggregates, which form senile plaques; and amyloid deposition near brain vessels resulting in inflammation, oxidative stress, and loss of neurons and synapses. Epidemiological studies show an increased risk of developing AD for the female gender (*Alzheimers Dement*, 2016). In fact, in AD cognitive impairment is greater in women than in men at the same stage of the disease, probably due to reduced estrogen levels in post-menopausal women (Vest et al., 2013). Studies in transgenic mice, models of the disease, suggest that females are more susceptible to the hippocampal stress response than males, indicating a greater vulnerability to tau (Sotiropoulos et al., 2015) and Aβ pathologies (Devi et al., 2010). Furthermore, the absence of ovarian hormones increases the already age-induced phosphorylation of tau in the rat hippocampus (Picazo et al., 2016). Still, more and more studies accentuate the estrogen-mediated neuroprotective role in neuroinflammation and neurodegenerative disorders. Indeed, estrogens enhance cell survival by inhibiting neurotoxic stimuli through their antioxidant activity(Nikiand Nakano, 1990). Recently, there has been increasing emphasis on the role of microRNAs (miRNAs) in neurodegenerative diseases; in fact, Xiong and colleagues (Xiong et al., 2015) observed a novel effect of miRNA-218. Specifically, they observed an opposite role for ERα and ERβ, two major estrogen receptors, in AD in regulating tau protein phosphorylation. Indeed, ERα increases the expression of miRNA-218, which acts inthe down-regulation of protein tyrosine phosphatase-α (PTPα). This inhibition leads to abnormal hyperphosphorylation of tyrosine by GSK3β (resulting in the activation) and protein phosphatase 2A (resulting in inactivation). The effect of increased miRNA-218 results in increased phosphorylation of tau. In contrast, ERβ acts by inhibiting miRNA-218, resulting in increased tau dephosphorylation.

In our laboratory, female mice expressing human tau protein (MAPT) and knockout for the murine gene were used to analyze the effects of estradiol. Our data showed that Aβ42 monomers, inoculated intracerebroventricularly, produced the pathological conformational changes and hyperphosphorylation of tau protein in ovariectomizedmale or female mice but not in the control female. Treatment of ovariectomized females with replacement estradiol protects against the pathological conformation of tau.
The hypothesized protective mechanism of estradiol is mediated by both its antioxidant activity and its ability to modulate the expression of miRNA-218 linked tau phosphorylation. To clinically correlate the results obtained, we measured miRNA-218 levels initially in CSF, then in plasma of AD patients, in MCI (mild cognitive *impairment*) patients with mild cognitive impairment, and in the control population, by *Real Time* RT-PCR, considering differences in gender and disease stage. The data revealed that miRNA-218 is more highly expressed in

patients with MCI and AD in both genders.
Interestingly,when analyzing only female gender, miRNA levels are double in women with AD compared with their control gender.
To date, there are several biomarkers for AD diagnosis, and each type of biomarker could characterize the pathogenesis. We want to better describe the role of miRNA-218 in Alzheimer's disease pathology and investigate whether miRNA-218 could be a possible biomarker to consider it as a strategic target for developinga therapeutic strategy.

c. General aim and integration with mission of the Institute

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

The protective role of estrogen against neurodegeneration and neuroinflammation is widely demonstrated, but the mechanism by which estrogen acts remains to be further investigated. We have recently developed a mouse model of AD that mimics the typical pathologicalevolutionofAD(ß-amyloid;humantauexpression):the5xTg-AD/hTauTg double transgenic. This project will focus not only on patients but also on animal experimentation, using this very novel mouse model, with the aim of developing a new therapeutic approach.
The specificobjectivesthisprojectareasfollows:

1. To dose miRNA-218 expression in patients to characterize its modulation in a larger population by analyzing its levels by *Real Time* RT-PCR in patients' plasma. Preliminary data obtained from patients' plasma allow us to monitor miRNA concentration over time, using a biological sample that is easier to obtain and less invasive.
2. In conjunction with the analysis of these samples, we will begin experiments on 5xTg-AD/hTauTg animals. Based on the previously obtained and already published results from our laboratory (Guglielmotto et al. 2019), we will use both male and female mice, and the latter will be ovariectomized to simulate the lack of estrogen. Following the analysis of the amount of miRNA218 in the blood of mice, we will artificially inoculate an antisense miRNA-218 into mice by intracerebroventricular injection, to inhibit the function of miRNA. In the future, we may propose a spray drug containing this miRNA so that it can be taken easily and can pass the blood-brain barrier.
   In conclusion, the goals of this proposal are to find a correlation between miRNA-218 expression and AD and to discover a new biomarker already present in the plasma of MCI patients to find a possible therapeutic approach to reduce disease progression.
   MiRNA218 in the blood of mice, we will artificially inoculate an antisense miRNA-218 into mice by intracerebroventricular injection, to inhibit the function of miRNA. In the future, we may propose a spray drug containing this miRNA so that it can be taken easily and can pass the blood-brain barrier.

In conclusion, the goals of this proposal are to find a correlation between miRNA-218 expression and AD and to discover a new biomarker already present in the plasma of MCI patients to find a possible therapeutic approach to reduce disease progression.

e. Unique features of the project research

The primary neuropathological events of Alzheimer's disease and all other forms of dementia involve the aberrant formation of pathological protein species. Although science has provided the necessary tools to unravel the mechanisms underlying the synthesis, modification and precipitation of these proteins, the pathogenesis of brain tissue damage begins many years before the onset of neurological signs. The research conducted by our group is aimed at the discovery of predictive plasma biomarkers that would allow early identification of the disease. We believe that a preventive diagnostic approach may be useful in both prognostic and therapeutic settings. In addition, almost all the known cases of Alzheimer's disease cannot be traced to an unambiguous cause, so we study those environmental and behavioral factors that may determine neuronal damage and promote the onset of cognitive impairment over time.

Fondazione Cavalieri Ottolenghi

Neuroscience Institute Cavalieri Ottolenghi

Internal Annual Report 2024

Laboratory name: **Neurophysiology of neurodegenerative diseases**

1. LABORATORY DESCRIPTION – PERSONNEL:

Principal Investigator
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2. CURRENT GRANTS

Starting-end date	Project Title and ID	Beneficiary	Funding Program/Agency	Role of the unit	Overall AmountFunded	Managed by FCO/UNITO
2023-2025	Matrix Metalloproteinase-9 and PeriNeuronal Nets: new therapeutic targets for Fragile X Syndrome	Filippo Tempia	PRIN	component of Research Unit	€ 51.445	€ 0,00
01/10/23-31/10/25	Atassia Telangiectasia: identificazione di meccanismi di neurodegenerazione affrontabili con trattamenti farmacologici	Eriola Hoxha	CRT	PI	€ 30.000	FCO
01/08/2024 - 31/01/2026	Collaboration related to the link between the role of NURR1 in physiological and pathological conditions and the effects of various Immunic compounds	Francesca Montarolo	Immunic Therapeutics	PI	€ 250.000	FCO

3. SCIENTIFIC ACTIVITIES IN 2024

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
• others	
Organizational activities and responsibilities at NICO:	Group Leader of Neurophysiology of Neurodegenerative Diseases; Director of the NICO Animal Facility
Technology transfer achievements (patents, etc.):	Patent n. 102022000024564 released on 04/11/2024, 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 -Prof. Maximiliano Jose Nigro, (Dept. of Language and Literature), Kavli Institute for Systems Neuroscience, NTNU, Trondheim, NO
• Editorial duties:	Editor of Frontiers in Aging Neuroscience; Member of the editorial board of Frontiers in Cellular and Molecular Mechanisms of Brain-aging
• others	Referee for the grant agency National Science Center Poland (NCN) (PRELUDIUM 23).
Organizational activities and responsibilities at NICO:	Responsible for the water ultrapurification systems at NICO
Technology transfer achievements (patents, etc.):	Patent n. 102022000024564 released on 04/11/2024, with Filippo Tempia.

Francesca Montarolo, postdoc fellow

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	- Prof. Gabriele C. De Luca, (Nuffield Dept, of Clinical Neurosciences), University of Oxford, London, UK.

• Invited talks:	*NUclear Receptor-Related 1 protein (NURR1) in Multiple Sclerosis (MS).* September 10th, 2024, in the frame of the Immunic's Onsite Multiple Sclerosis R&D Day in New York City, USA. Invited oral communication. *Atm deficiency is associated with motor impairment and dysfunction of cerebellar circuitry in mice.* September 13th, 2024, in the frame of the 74° national meeting of the Italian Society of Physiology (SIF) in Rome, Italy. Abstract selected for oral communication.
• Editorial duties:	na
• others	Referee for the grant agency National Science Center Poland (NCN) (OPUS-24). Referee for the grant French Multiple Sclerosis Research Society (ARSEP Foundation). Referee for the grant agency Fondazione ItalianaSclerosiMultipla (FISM).
Organizational activities and responsibilities at NICO:	na
Technology transfer achievements (patents, etc.):	na

ROMINTO Anita Maria, PhD student

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	-Prof. Maximiliano Jose Nigro, (Dept. of Language and Literature), Kavli Institute for Systems Neuroscience, NTNU, Trondheim, NO
• Invited talks:	
• Editorial duties:	
• others	*Altered GSK3 and prefrontal cortex activity in a mouse model of depression.* Poster. November 12th, 2024. SINS National Meeting of PhD students in neuroscience.
Organizational activities and responsibilities at NICO:	na
Technology transfer achievements (patents, etc.):	na

BERRINO Luna, research fellow

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	na
• Invited talks:	na
• Editorial duties:	na
• others	na
Organizational activities and responsibilities at NICO:	na
Technology transfer achievements	na

4. Research activity in 2024

a. Summary

In an animal model of depression, in the medial prefrontal cortex we found a deficit in action potential generation, due to a more pronounced frequency adaptation. In an animal model of AT, we found novel electrophysiological alterations of Purkinje cell activity. We found that the enzyme Elovl5 is necessary for proper neurotransmitter release and synaptic vesicle cycle. Since August, we established a new financed collaboration with Immunic Therapeutics in order to study the effect of a class of NURR1 agonists in neuro-inflammation.

b. Background and rationale

1.The molecular mechanisms of depression are not clearly understood, and antidepressant drugs have a low rate of efficacy. The development of more effective drug is hindered by the lack of knowledge of the neuronal alteration present in depression. The medial prefrontal cortex (mPFC) is a main hub controlling the circuit involved in stress and depression. Moreover, functional neuronal alterations are associated with dysregulation of signaling pathways. The kinase GSK3 is hypothesized to be a pivotal regulator of neuronal excitability and synaptic transmission. The discovery of the role of GSK3 in depression 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.

3. Neurotransmitter release and synaptic vesicle cycle require specific lipidic composition of presynaptic and vesicle membranes. Phospholipids with long-chain acyl groups are necessary to allow membranes to have correct physical properties necessary for their role in synaptic transmission. Elovl5 is a key enzyme for the elongation of polyunsaturated fatty acids (PUFAs) beyond 18 carbon atoms. Elovl5 deletion in mice or mutation in humans causes cerebellar motor deficits. In the central nervous system, deletion of *Elovl5* increases upstream 18 and 20 carbon atoms PUFAs, decreases long chain PUFAs and increases saturated and monounsaturated fatty acids. Our hypothesis is that Elovl5-dependent PUFAs have an important role in the proper function of the synaptic vesicle cycle.

4. Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system characterized by neuro-inflammation demyelination, and neurodegeneration without definitive cure. Our previous studies demonstrated a reduced level of *NURR1* (*NUclear Receptor-Related Protein 1*) gene expression in the blood of patients with MS compared to healthy controls. NURR1 is a transcription factor that exerts an anti-inflammatory role in the peripheral blood and in the central nervous system. A class of disease-modifying treatments recently discovered by Immunic Therapeutics and proposed to manage MS also act through a not-well described modulation of NURR1.

c. Objectives

Aim 1. Neuronal mechanisms of depression. The aim of the research was to investigate, in pyramidal neurons of the mPFC, the alterations in action potential firing induced by the development of a depressive-like behavior following the chronic social defeat stress paradigm.

Aim 2. Functional alterations in cerebellar Purkinje cells in an animal model of AT. The aim was to find differences in action potential activity and/or in synaptic signaling.

Aim 3. Role of Elovl5 in synaptic vesicle cycle. The aim was to detect deficiencies in synaptic transmission and to identify the mechanism responsible. The final objective was to understand which phases of the synaptic vesicle cycle require Elovl5-dependent PUFAs.

Aim 4. Role of NURR1 in neuro-inflammation. The aim was to dissect the role of NURR1 by means of *in-vitro* and *in-vivo* systems of inflammation using molecules able to modulate its function.

d. Results

Aim 1. Neuronal mechanisms 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. Pyramidal neurons of the mPFC were recorded by patch-clamp in acutely prepared tissue slices. In contrast with previous findings with different induction protocols, using indirect techniques and with contrasting results, we found ...

Aim 2. Functional alterations in cerebellar Purkinje cells in an animal model of AT. Action potentials were evoked in cerebellar Purkinje cells by direct depolarization via the patch pipette. We found that such evoked firings showed aberrantly higher frequency compared to control animals. This might be a mechanism of the early signs of ataxia in AT. While the parallel fiber-Purkinje cell synapse was intact, we found that the climbing fiber-Purkinje cell synapse showed a more pronounced paired pulse depression, which is a form of synaptic plasticity in which the calcium-dependent release of neurotransmitter is involved.

Aim 3. Role of Elovl5 in synaptic vesicle cycle. In mice with deletion of Elovl5, basal synaptic transmission was preserved, but the recovery from paired pulse depression of the climbing fiber synapse lacked the fast phase, suggesting a deficit in the replenishment of the readily releasable pool of synaptic vesicles. Parallel fiber synaptic transmission showed a deficit at relatively high frequencies of 50 and 100 Hz, which are within the physiological range. This deficit could be attributed to a slower replenishment rate of the readily releasable pool. Therefore, at both synapses, Elovl5-dependent lipids are necessary to maintain synaptic signaling. Moreover, the synaptically induced suppression of excitation (SSE)., which is based on endocannabinoid release, in Elovl5 knockout mice had a shorter duration, in line with a role of Elovl5. In conclusion, the shift in PUFA lipidic species caused by the absence of Elovl5, in the cerebellar cortex is responsible for specific deficits in neurotransmitter release, associated with motor impairment.

Aim 4. In the first months of the financed project we prepared and obtained the authorization by the Italian Ministry of Health to perform the animal experimentation.

e. Advancement in the field

Our results on neuronal alterations in the animal model of depression are the first hint to the neural mechanisms responsible for this psychiatric disorder. They are a starting point to investigate the role of Gsk3 and Fgf14, which might become novel target of a new generation of antidepressant drugs.

The finding of deficits in action potential firing and of synaptic transmission in the animal model of AT are completely novel, and they are an indispensable knowledge to design a therapy.

The role of the lipidic composition of vesicle and presynaptic membranes is still undefined. Our results on Elovl5-dependent PUFA are an important milestone to understand the specific role of this class of lipids in the synaptic vesicle cycle.

Our findings on NURR1 and neuro-inflammation will open up future therapeutic strategies to treat MS by acting directly on the central nervous system and not just the immune system, as is generally the case with most disease-modifying treatments.

f. Publications[1]

Three submitted manuscripts.

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

Starting from our results on neuronal firing in animal model of depression, we plan to investigate the role of Gsk3 and Fgf14 in such functional alterations. In the animal model of AT, in which we found disfunction in action potentials and synaptic transmission, we plan to search for alterations in calcium signaling. In another line of research, we plan to study the role of Elovl5 in the conduction of action potentials in the central nervous system. Also the role of NURR1 in neuro-inflammation will be investigated.

b. Background and Significance

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. Fgf14 is a cytoplasmic protein that regulated the function of voltage-dependent sodium channels. Our findings on the resilience of Fgf14 knockout mice to depression will be a starting point to investigate the involvement of Fgf14 in the pathogenesis of depression.

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. In the last year the research was focused on the early alterations in PC physiology, including action potential firing and postsynaptic events. However, indirect evidence suggests an important involvement of deficits in calcium signaling.

3.Neurotransmitter release and synaptic vesicle cycle require specific lipidic composition of presynaptic and vesicle membranes. Phospholipids with long-chain acyl groups are necessary to allow membranes to have correct physical properties necessary for their role in synaptic transmission. Elovl5 is a key enzyme for the elongation of polyunsaturated fatty acids (PUFAs) beyond 18 carbon atoms. Elovl5 deletion in mice or mutation in humans causes cerebellar motor deficits. In the central nervous system, deletion of *Elovl5* increases upstream 18 and 20 carbon atoms PUFAs, decreases long chain PUFAs and increases saturated and monounsaturated fatty acids. We previously ì showed that Elovl5-dependent PUFAs are important for proper action potential conduction in peripheral nerves. However, the role of Elovl5 in axons of the central nervous system is still unknown.

4. Multiple Sclerosis (MS) is an autoimmune disease of the central nervous system characterized by neuro-inflammation, demyelination, and neurodegeneration, without definitive cure. Our previous studies demonstrated a reduced level of *NURR1* gene expression in the blood of patients with MS compared to healthy controls. A class of disease-modifying treatments recently discovered by Immunic Therapeutics and proposed to manage MS also act through a not-well described modulation of NURR1.

c. General aim and integration with mission of the Institute

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 MS. 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 basic mechanisms, which are altered in neurological disorders.

d. Specific objectives and strategies

Aim 1: Role of Gsk3 and Fgf14 in the control of neuronal functional alterations caused by induction 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 the last year we found that some parameters of action potential evoked firing are altered in CSDS. Unpublished data also suggest that synaptic transmission is altered in the mPFC. In the next year we plan to start with an evaluation of synaptic transmission following CSDS. 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: The main aim of this phase of research on the neuronal mechanisms of AT is to find dysregulated calcium signals exploiting our animal model. The alteration that we found in the short-term plasticity of the climbing fiber-Purkinje cell synapse suggests a problem with calcium signaling. We plan to start with an evaluation of the basal calcium level in Purkinje cells. Calcium signal evoked in Purkinje cell by depolarization or by synaptic activation will be studied in vitro in cerebellar slices. Spontaneous calcium signals produced by climbing fiber activity will be studied in vivo by two photon microscopy.

Aim 3: The aim of this research is to determine the effects of Elovl5 deficiency on action potential conduction along axons in the central nervous system.

Aim 4: The aim is to analyze the role of NURR1 during neuro-inflammation using a murine model of MS, such as the Experimental Autoimmune Encephalomyelitis (EAE) and treatments with molecules capable of modulating the function of the transcription factor.

e. Unique features of the project research

1. By the experiments of Aim1 we expect to identify Gsk3 and Fgf14 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 functional neuronal alterations of pyramidal neurons of 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 determine the role of the Gsk3 pathway in the induction of the alterations of action potentials and synaptic transmission. This would open the way to the development of new drugs with a better efficacy relative to current therapies.

2. In ataxia-teleangiectasia, calcium dysregulation might be a major mechanism of Purkinje cell dysfunction and ultimately cell degeneration. The identification such mechanisms would open the way to design new treatments to rescue Purkinje cells and prevent ataxia in patients with AT.

3. A deficit in action potential conduction, especially in cerebellar neurons, could disrupt the precise timing of signals necessary for motor control. Moreover, the results might provide new knowledge about the role of the lipidic composition of myelin for the optimization of action potential conduction at high velocity.

4. In MS, NURR1 could influence neuro-inflammatory aspects of the disease. The dissection of this mechanism could open up future therapeutic strategies to treat MS by acting directly on the central nervous system.

f. **Methodology**

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 2024

Laboratory name: **Brain development and disease**

1. LABORATORY DESCRIPTION – PERSONNEL:

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

Stanga, Serena, Associate Professor, PhD, 03/06/1983, +390116706632, serena.stanga@unito.it, Ageing and Alzheimer's disease (AD), motor neuron diseases (ALS and SMA), molecular neuroscience, primary neuronal cultures, mitochondrial dysfunctions

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

Schellino, Roberta, Assistant Professor RTD-B, PhD, 11/02/1985, +390116706632, roberta.schellino@unito.it, Neurogenesis, motor neuron and neuromuscular diseases, Huntington's disease, aging, green therapy, histology, confocal imaging, cell tracing, behavior

Pacca, Paolo, Assistant Professor RTD-A, MD, PhD, 23/06/1984, +390116706632, paolo.pacca@unito.it, Glymphatic system in spinal cord injury and motor neuron diseases, Cell culture, muscular atrophy, aging, Robotic technology for elderly care

Menduti, Giovanna, II level technologist, PhD, 14/04/1991, +390116706632, giovanna.menduti@unito.it, Cellular and molecular neurobiology, spinal muscular atrophy, drug repositioning

Bartesaghi, Luca, Post-doc research fellow (since September 2024), PhD, 28/12/1980, luca.bartesaghi@unito.it, +390116706632, Myelinating Glia, developmental neuroscience, light-microscopy, 3D modelling, molecular biology, cell culture, animal experimentation

La Rocca, Federica, Post-doc fellow (since December 2024), PhD, 18/07/1994, +390116706632, federica.larocca@unito.it, Spinal muscular atrophy, neuromuscular diseases

Mattioni Anna, Post-doc fellow (since October 2024), PhD, 02/12/1986, +390116706632, anna.mattioni@unito.it, Ageing and Alzheimer's disease, cell culture, organoids, iPSCs, molecular and cellular biology, correlative microscopy

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

Chasseur, Silvia, Research fellow, Master degree in Biotechnology for Neuroscience, 01/11/1999, +393459535393, silvia.chasseur@unito.it, Spinal cord injury, bioprinting, cell culture, cellular and molecular analysis

Chicote, Javier, Research fellow, Bachelor degree, 18/01/1987, +390116706632, javier.chicotegonzalez@unito.it, Ageing and Alzheimer's disease, brain iron metabolism, histological and molecular analysis

Iezzi, Giulia, Research fellow (since July 2024), Master degree in Medical Biotechnology, 05/10/1999, +390116706632, giulia.iezzi@unito.it, Chemotherapy-induced cognitive impairment, spinal muscular atrophy, histological analysis

Tuninetti, Alessandra, Research fellow (since November 2024), Master degree in Animal Biotechnology, 13/06/1998, alessandra.tuninetti@unito.it, Glymphatic system in spinal cord injury and motor neuron diseases, biomarkers

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

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

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, electron microscopy

Dotta, Sofia, PhD student, Master degree in Biotechnology for Neuroscience, 21/09/1999, +390116706632, sofia.dotta@unito.it, pain research during aging, embryonic and adult DRG culture, neurite outgrowth assay, molecular biology and imaging

Ferrero, Clelia, PhD student, Master's Degree in Neurobiology, 30/09/1997, +390116706632, clelia.ferrero@unito.it, Amyotrophic lateral sclerosis, Frontotemporal Dementia, stem cells, iPSCs, organoids

Nicorvo, Ersilia, PhD student, Master degree in Neuroscience, 31/05/1995, +390116706632, ersilia.nicorvo@unito.it, Spinal muscular atrophy, drug reposition, cell culture, iPSCs, glioblastoma, neuron-tumor crosstalk

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

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

Scimia, Noemi, PhD student (since November 2024), Master degree in Biotechnology for Neuroscience, 25/02/1999, +390116706632, noemi.scimia@unito.it, Aging and Alzheimer's disease, motor neuron diseases (ALS and SMA), brain iron metabolism, histological and molecular analysis

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

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
2024	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
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
2024-2025	Daisy&Ron	Vercelli A	PNRR MUR - Anthem	Coordinator	578.595	UNITO – Teoresis - Intravides
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
2022-2025	NODES - INNDIANA	Boido M.	PNRR	P.I. of the research unit	60,000 €	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-	A 3D Bioprinted	Boido M.	PRIN 2022	Coordinator	61,711 €	UNITO

2025	spinal cord Model to reach functional meaningful and clinically translatable regeneration					
2024-2025	A multiscale imaging approach to study early alterations in an Alzheimer cerebral organoid (MULTIMALZ)	Boido M.	PNRR MUR	Coordinator (Co-PI Roberta Schellino)	280.960 €	FCO
2024-2027	REACT: Rebalancing the Epitranscriptional landscape as an AdvanCed Therapy in SMA	Boido M.	CURE SMA 2024	P.I. of the research unit	12.420 $	FCO
2024-2027	InibiREl'infiAmmazione per Contrastare l'aTrofIamuscOlarenel l'aNziano (REACTION)	Boido M.	Fondazione CRT (progetti interdipartimentali)	P.I. of the research unit	14.950	UNITO
2021-2024	NODES - TINCARE	Calì C.	PNRR	P.I. of the research unit	60.000 €	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
2022-2024	Braccio di ferro con la demenza: ferro e mitocondri come nuovi target contro la Malattia di Alzheimer	Stanga S.	Fondazione CRT	Coordinator	20,000 €	FCO
2023-2025	Targeting mACONITASE and KIF5a to rescue mitochondrial mobility and function for the treatment of motor neuron diseases	Stanga S.	PRIN 2022	P.I. of the research unit	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.	Minoryxtherapeutics	Coordinator	60,000 €	FCO
2024-2026	Evaluation of the effect of leriglitazoneon cognitive behaviour in	Stanga S.	Minoryxtherapeutics	Coordinator	34,800 €	FCO

	a murine model of Alzheimer's disease					

3. SCIENTIFIC ACTIVITIES IN 2024

Alessandro Vercelli, PI

Supervised PhD students:	A. Caretto (co-tutorship with M. Boido), S. Dallere, G. Pavarino, S. Dotta, Ricardo Madeira
Honors, prizes, awards:	
Outreach activities	
● International collaborations:	G. Aumayr (Austria), M. Summers (Australia), Makoto Sato (Osaka University, Japan), Javier De Felipe (Cajal Institute), Serenella Tolomeo (Singapore), Helena Vieira (Lisboa, Portugal), PharmafoxTherapeutics
● Invited talks:	May 2024 Frontiers in Neuroscience, Buzios (BR)
● Science communication:	Scientific contribution within the exhibition "The Mountain Touch - Un viaggio nella natura che cura", Museo delle Scienze di Trento (MUSE), Trento, Italy (28/07/2024 – 17/11/2024) Interview "Le neuroscienze in Italia" within the television program "Tu Chiami, TL Risponde" (UP Salute), TeleLombardia, Milan, Italy (15/07/2024)
● Editorial duties:	Associate Editor Frontiers Ageing Neuroscience
● others	Institutional visits in Brazil, Singapore, South Korea and US (Boston area) to establish collaborations with NICO and UNITO; followup of previous visits in Israel and Japan
Organizational activities and responsibilities at NICO:	Scientific Director
Speakers invited:	Filippo Sean Giorgi (Univ. Pisa); Makoto Sato (Univ. Osaka); Alessandro Usiello (Univ. Campania L. Vanvitelli); Helena Vieira (Univ. Lisbon); Manuel Valiente (CNIO, Madrid)
Otherorganizational activities:	
Workshops, Schools or Conferences organized:	
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, Ana Alexandra Flores da Silva (co-tutorship with A.C. Cristovao, Univ. Beira Interior)
Honors, prizes, awards:	na
Outreach activities	
● International collaborations:	Prof. Artero (Univ. Valencia), Prof. Soler (University of Lleida, Spain), PharmafoxTherapeutics 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), Dr Rico Schieweck (University of Luxembourg)
● Invited talks:	Invited talks at meetings:

	"Beyond motor neurons: skeletal muscle contribution in Spinal Muscular Atrophy". Towards an Italian SMA Connectome - National Meeting for Innovation and Shared Research, Roma, 26/01/2024 "Spinal cord injury and mesenchymal stem cell transplantation: what we have learned from preclinical models". Workshop "Experimental tissue models as sustainable tools to protect human health", Politecnico di Torino. Turin, Italy, 29/04/2024 "Spinal muscular atrophy: more than a motor neuron disease". 5th International Symposium Frontiers in Neuroscience, Buzios, Brazil (16/05/2024) "Utilizzo di modelli murini SMA: esempi di studi preclinici per approfondire la patogenesi e definire nuove strategie terapeutiche". Workshop "SMA: dalla rivoluzione terapeutica allo screening neonatale", CEINGE, Naples, Italy (24/06/2024) Invitation upon selection at meetings: Boido M, Schellino R, Menduti G, Caretto A, Vercelli A. Morphological alterations of cortical projection neurons and interneurons in a murine model of Spinal Muscular Atrophy. 21st Congress of the International Federation Association of Anatomists (IFAA), Gwangju, South Korea (05-08/09/2024) Menduti G Konieczny P, Berenge-currias N, Martinat C, Artero R, Boido M. "Drug repositioning as a therapeutic strategy for Spinal Muscular Atrophy treatment". 77th national meeting of the Italian Society of Anatomy and Histology (S.I.A.I.). Genoa, Italy (12-14/09/2024) Boido M, Menduti G, Rasà DM, Stanga S, Artero R, Martinat C, Di Schiavi E, Vercelli A. Drug repositioning for spinalmuscularatrophy. XXXIV National Conference of the Italian Group for the Study of Neuromorphology (GISN), Catania, Italy (22-23/11/2024)
• Science communication:	Regional coordinator (Piedmont) of Olympics in Neuroscience; Regional stage, Turin (09/03/2024) Scientific contribution within the exhibition "The Mountain Touch - Un viaggionella natura checura", Museo delleScienze di Trento (MUSE), Trento, Italy (28/07/2024 – 17/11/2024) Interview "Le neuroscienze in Italia" within the televisionprogram "Tu Chiami, TL Risponde" (UP Salute), TeleLombardia, Milan, Italy (15/07/2024) Caffè scientifico "Atrofia Muscolare Spinale: nuove terapie per la salute dei bambini", within the "EuropeanResearchers' Night", Turin, Italy (28/09/2024) Conference "Un viaggio nella natura", Festival dell'Innovazione e della Scienza, Settimo T.se (TO) (13/10/2024). Round Table "Reflecting on Systemic Interventions", within the workshop "Mental Health in Academia and Virtuous Academic Culture", Turin, Italy (17/10/2024) Speech as Alba Representative within the "GiornataRegionaledell'Inclusione", organized by UP Salute,

	Milan, Italy (19/10/2024)
• Editorial duties:	Associate Editor for Frontiers in Medical Engineering Review editor Frontiers in Aging Neuroscience
• 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 Representative of the Italian Society for Neuroscience and NICO to the ALBA Network Poster at meeting: Boido M., Schellino R., Vrijbloed J.W., Fariello R.G., Vercelli A. The biological ActR-Fc-nLG3, synergistically acting on myostatin and agrin pathways, reinforces neuromuscular stability and increases motor endurance in old mice. Neuroscience 2024, Chicago, USA (05-09/10/2024)
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:	Elia Di Schiavi (CNR Naples); Piera Smeriglio (Center of Research in Myology, Sorbonne University, Paris)
Otherorganizational activities:	CEO of S&P BRAIN SRL
Workshops, Schools or Conferences organized:	Organization of the III edition of the Workshop "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies", with Prof. Stanga, Turin, Italy (08-09/11/2024)
Technology transfer achievements (patents, etc.):	na

Corrado Calì, Associate Professor

Supervised PhD students:	M. F. Veloz Castillo, V. Chiappini
Honors, prizes, awards:	Best paper award at IEEE WEB3D conference, Guimares, Portugal, September 2024
Outreach activities	
• International collaborations:	Pierre Magistretti (KAUST, Saudi Arabia), Marco Agus (Hamad Bin Khalifa University, Qatar), Markus Hadwiger (KAUST, Saudi Arabia)
• Invited talks:	Zeiss EM Workshop, Lausanne (14/01/2024) "General Aspects in the Evolution of Glia", Evolutionary roots of human brain disease conference, Luxembourg (22/02/2024) "Stabilization of synapses following Lactate injection", Clinatec, Grenoble, France (22/5/2024) "Lactate from astrocytic glycogen is necessary for stabilization of synapses following learning", Connectomics conference, Berlin, Germany (18/6/2024) "Ultrastructural localization of astrocytic glycogen and its relationship to synaptic arrangement", Linking brain structure & function at the nanoscale: an interdisciplinary workshop,

	Lyon, France (27/09/2024) "NeuroVerse—Immersive exploration of 3D ultrastructural brain reconstructions for education and collaborative analysis", Web3D conference, Guimares, Portugal (28/09/2024)
● Science communication:	Festival Digitale Popolare 2024, III Edizione (04/10/2024)
● Editorial duties:	Associate editor of Advanced Technology in Neuroscience
● others	Member of the review panel of EIC Pathfinder program; Member of the review panel of the Brayn Starting Grant; Responsible for Erasmus program of Biotechnology for Neuroscience course
Organizational activities and responsibilities at NICO:	Responsible for the Gemini 460 SEM Volutome system
Speakers invited:	Pierre Magistretti (KAUST, Saudi Arabia)
Otherorganizational activities:	President of Intravides SRL
Workshops, Schools or Conferences organized:	Member of the organizing committee of FISV 2024 congress and moderator of Epigenetic session, Padua, Italy (18-20/09/2024)
Technology transfer achievements (patents, etc.):	na

Serena Stanga, Associate Professor

Supervised PhD students:	Noemi Scimia
Honors, prizes, awards:	na
Outreach activities	
● International collaborations:	Prof. P. Kienlen-Campard (UCLouvain, Bruxelles Belgium); Dr. E. Auduard and Prof. F. Piguet (Inserm, Paris, France); Prof. A. Prochiantz and Dr. A. Di Nardo (College de France, Paris, France); Prof. C. Svendsen, Dr. D. Lall and Dr. F. Diaz (Cedars-Sinai, Los Angeles, USA); Minoryx Therapeutics (Spain, Belgium)
● Invited talks:	Invitedseminars: Seminar at UniversitàdegliStudi di Napoli Federico II "Brain iron distribution and mitochondrial features in the early phases of amyloidosis in 5xFAD mouse model", Naples, Italy (10/10/2024) Invitation upon selection at meetings: "Aconitase as a marker of early pathological state in SMA: data from spinal cord and fibroblasts" 4th SMA EUROPE, Ghent, Belgium (14-16/04/2024) "A role for dysfunctionalmitochondria in ALS" 34th Congress Gruppo Italiano per lo Studio della Neuromorfologia (GISN), Catania, Italy (22-23/11/2024)
● Science communication:	"Metti in gioco i tuoi neuroni" with E. Signorino, Area Play, UNIGHT 2024, Turin, Italy (28/09/2024)
● Editorial duties:	External expert for the European Commission, Evaluations for HORIZON MSCA 2023-DN-01 Evaluator for the "Premio GiovediScienza 2024" Evaluator for the Joint Programming Neurodegenerative Disease Research (JPND) 2024

	Review editor for Alzheimer's Research & Therapy
● others	Posters at meetings: Mezzanotte M, Chicote J, Boido M, Vercelli A, StangaS."Brain iron accumulation: a shared hallmark of aging and Alzheimer's disease?" 77th national meeting of the Italian Society of Anatomy and Histology (S.I.A.I.). Genoa, Italy (12-14/09/2024) Stanga S, Lall D, Diaz F, Di Lullo E, Mezzanotte M, Chicote J, Hernandez S, Sansa A, Prochiantz A, Svendsen C.N.A Role for dysfunctionalmitochondria in ALS. Workshop "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies" (III edition), Turin, Italy (08-09/11/2024)
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 Initiator with S. Bovetti of the NICO NEWS online monthly since 2024
Speakers invited:	Antonella Di Bello (3Brain)
Otherorganizational activities:	Organization of the Lab meetings 2024 of the group: Brain development & disease
Workshops, Schools or Conferences organized:	Organization of the III edition of the Workshop "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies", with Prof. Boido, Turin, Italy (08-09/11/2024)
Technology transfer achievements (patents, etc.):	na

Letizia Marvaldi, Assistant Professor RTD-B

Supervised PhD students:	S. Dotta (co-tutorship with A.Vercelli)
Honors, prizes, awards:	Rita Levi Montalcini Fellowship (MIUR)
Outreach activities	
● International collaborations:	Dr. Franziska Rother (MDC Berlin; Germany), Prof. Michaela Kress (Medical University Innsbruck, Austria), Prof. Mike Fainzilber and Dr. Ida Rishal (Weizmann Institute of Science, Rehovot, Israel)
● Invited talks:	Pain mechanism and Therapeutics Conference "Importin alpha3 controls age-dependent axonal branching by modulating ribosomal protein expression", Verona, Italy (17-22/05/2024)
● Science communication:	na
● Editorial duties:	na
● others	na
Organizational activities and responsibilities at NICO:	Coordination for BioRenderlicense (2023-2025)
Speakers invited:	Dr. Marco Terenzio (OIST, Japan)
Otherorganizational activities:	na
Workshops, Schools or Conferences organized:	Member of the local organizing committee at workshop "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies" (III edition), Turin, Italy (08-09/11/2024)

Technology transfer achievements (patents, etc.):	na

Roberta Schellino, Assistant Professor RTD-B

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
● International collaborations:	J. Willem Vrijbloed (PharmafoxTherapeutics AG); R. Fariello (PharmafoxTherapeutics AG)
● Invited talks:	Invitation upon selection at meetings: Schellino R, Caretto A, Iezzi G, Boido M., Vercelli A. "Time-course characterization of cortical projection neuron's alterations and development in delta7 SMA mice". 4th International Scientific & Clinical Congress on Spinal Muscular Atrophy, SMA Europe. Ghent, Belgium (14-16/03/2024) Schellino R, Caretto A, Iezzi G, Boido M., Vercelli A."Time-dependent characterization of cortical projection neuron's alterations and development in a murine model of Spinal Muscular Atrophy". 77th national meeting of the Italian Society of Anatomy and Histology (S.I.A.I.). Genoa, Italy (12-14/09/2024) Schellino R, Caretto A, Iezzi G, Boido M., Vercelli A."Time-dependent alteration of projection neurons due to lack of SMN protein significantly impacts the cortical cytoarchitecture in a murine model of Spinal Muscular Atrophy". Neuroscience 2024, Chicago, USA (05-09/10/2024) Invited speaker for the practical activity: "How to analyse the morphology and innervation of neuromuscular junctions, by image analysis software". Workshop "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies" (III edition). Turin, Italy (08-09/11/2024)
● Science communication:	Member of the local organizing committee at "OlimpiadidelleNeuroscienze", Turin, Italy (09/03/2024) Festival Women & the City: Women in STEM event (18-26/09/2024)
● Editorial duties:	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	na
Organizational activities and responsibilities at NICO:	Responsible for E800 Nikon Eclipse fluorescencemicroscope
Speakers invited:	Jeroen Pasterkamp (UMC, Utrecht Univ., The Netherlands)
Otherorganizational activities:	na
Workshops, Schools or Conferences	Member of the local organizing committee at workshop

organized:	"Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies" (III edition), Turin, Italy (08-09/11/2024)
Technology transfer achievements (patents, etc.):	na

Paolo Pacca, Assistant Professor RTD-A

Supervised PhD students:	na
Honors, prizes, awards:	National Scientific qualification (ASN) as associate, Academic Recruitment Field 05/H - Human anatomy, according to the Italian higher education system, in the call 2023/2025 (Ministerial Decree n. 1796/2023) for the disciplinary field of 05/H1 - Human anatomy
Outreach activities	
● International collaborations:	Helena Vieira (Lisboa, Portugal); J. Willem Vrijbloed (PharmafoxTherapeutics AG); R. Fariello (PharmafoxTherapeutics AG)
● Invited talks:	Invitation upon selection at meetings: Pacca P., Jhawar S.S., Snyderman C., Gardner P., J. C. Fernandez-Miranda. "Live Cadaver" Model for Internal Carotid Artery Injury Simulation in Endoscopic Endonasal Skull Base Surgery. 77th national meeting of the Italian Society of Anatomy and Histology (S.I.A.I.). Genoa, Italy (12-14/09/2024)
● Science communication:	Member of the local organizing committee at "OlimpiadidelleNeuroscienze", Torino, Italy (09/03/2024)
● Editorial duties:	na
● others	Poster at meeting: Pacca P, SS Jhawar, C Snyderman, P Gardner, Fernandez-Miranda JC. "Live Cadaver" Model for Internal Carotid Artery Injury Simulation in Endoscopic Endonasal Skull Base Surgery. First MorFuture 2024 conference on morphological studies between tradition and innovation. Turin, Italy (12-13/04/2024)
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Otherorganizational activities:	na
Workshops, Schools or Conferences organized:	Member of the local organizing committee at workshop "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies" (III edition), Turin, Italy (08-09/11/2024)
Technology transfer achievements (patents, etc.):	na

Giovanna Menduti, II level technologist

Supervised PhD students:	na
Honors, prizes, awards:	28/11/2023_Awarded with the "Disease Models & Mechanisms (DMM) Conference Travel Grant - CTG-DMM23111316 (The Company of Biologists)" to attend the 4th Scientific International Congress on Spinal Muscular Atrophy - SMA Europe (Ghent, Belgium,14–16/03/2024)

Outreach activities	
● International collaborations:	Prof. Artero (Univ. Valencia), Dr. Martinat (I-STEM, Corbeil-Essonnes, France)
● Invited talks:	na
● Science communication:	Member of the local organizing committee at “OlimpiadidelleNeuroscienze”, Turin, Italy (09/03/2024)
● Editorial duties:	na
● others	Posters at meetings: Menduti G, Beltrando G, Carosio M, Boido M*, Vercelli A*. “Unravelling the role of GABA signalling and metabolism (dys)regulation in Spinal Muscular Atrophy: results from SMAΔ7 mice cortex” (Poster and Flash Poster presentation), 4th International Congress on Spinal Muscular Atrophy, SMA Europe, Ghent, Belgium (14–16/03/2024) Menduti G, Beltrando G, Carosio M, Boido M*, Vercelli A*. “GABA signalling and metabolism (dys)regulation in SpinalMuscularatrophy: a new disease target atcorticallevel?, "MorFuture: gli studi di morfologia tra tradizione e innovazione", Turin, Italy (12-13/04/2024) Menduti, G., Ruatti C., Berenger-Currias N., JanuelC.,Pérez Gómez R. , Konieczny P., Artero R., Martinat C., Boido M. “A new effective therapy for Spinal Muscular Atrophy identified by drug repositioning: results in delta7 mice”, Workshop "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies” (III edition), Turin, Italy (08–09/11/2024)
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Otherorganizational activities:	na
Workshops, Schools or Conferences organized:	Organization of the First Congress of the "Amici dellaMorfologia" young Committee "MorFuture: glistudi di morfologiatratradizione e innovazione", Turin, Italy (12-13/04/2024) Member of the local organizing committee at Workshop "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies” (III edition), Turin, Italy (08–09/11/2024)
Technology transfer achievements (patents, etc.):	na

Anna Mattioni, Post-doc fellow

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
● International collaborations:	na
● Invited talks:	Oral presentation upon selection at the 2024 Women in Autophagy (WIA) annual symposium (28/10/2024)
● Science communication:	na
● Editorial duties:	na
● others	na

Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Otherorganizational activities:	na
Workshops, Schools or Conferences organized:	Member of the local organizing committee at Workshop "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies" (III edition), Turin, Italy (08–09/11/2024)
Technology transfer achievements (patents, etc.):	

Mariarosa Mezzanotte, Post-doc fellow

Supervised PhD students:	na
Honors, prizes, awards:	Winner of Borsa di Ricerca in the context of the grant "Post-Doctoral Fellowships - Anno 2024" funded by "Fondazione Umberto Veronesi" starting date 01/04/2024 until 31/03/2025
Outreach activities	
● International collaborations:	MinoryxTherapeutics (Spain, Belgium)
● Invited talks:	na
● Science communication:	Member of the local organizing committee at "OlimpiadidelleNeuroscienze", Torino, Italy (09/03/2024)
● Editorial duties:	na
● others	Posters at meetings: Mezzanotte M, Chicote J, Scimia N, Toce A, Stanga S. Brain iron dyshomeostasis and mitochondrial features' alteration in 5xFAD mouse model in the pre-pathological phase of Alzheimer's disease. 1° Congresso del comitato giovani degli Amici della Morfologia. "MorFuture" gli studi di morfologia tra tradizione e innovazione. Torino, Italy (12-13/04/2024); Mezzanotte M, Chicote J, Scimia N, Toce A, Rosano V, Stanga S. Brain iron and mitochondrial features in 5xFAD mouse. XXXIV Congresso Gruppo Italiano per lo studio della Neuromorfologia (GISN), Catania, Italy (22-23/11/2024)
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Otherorganizational activities:	na
Workshops, Schools or Conferences organized:	Member of the local organizing committee at Workshop "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies" (III edition), Torino, Italy (08-09/11/2024)
Technology transfer achievements (patents, etc.):	na

Silvia Chasseur, Research fellow

Supervised PhD students:	
Honors, prizes, awards:	na
Outreach activities	
● International collaborations:	na
● Invited talks:	na

● Science communication:	na
● Editorial duties:	na
● others	Poster at meeting: Chasseur S, Traldi C, Chiappini V, Tonda-Turo C, Boido M. "An in vitro 3D-bioprinted model of spinal cord as a tool in motor neuron disease research" Motor neuron diseases: understanding the pathogenetic mechanisms to develop therapies (III edition), Turin, Italy (08-09/11/2024)
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Otherorganizational activities:	na
Workshops, Schools or Conferences organized:	Member of the local organizing committee at Workshop "Motor neuron diseases: understanding the pathogenetic mechanisms to develop therapies" (III edition), Torino, Italy (08-09/11/2024)
Technology transfer achievements (patents, etc.):	

Javier Chicote, Research fellow

Supervised PhD students:	na
Honors, prizes, awards:	
Outreach activities	
● International collaborations:	MinoryxTherapeutics (Spain, Belgium)
● Invited talks:	na
● Science communication:	Member of the local organizing committee at "OlimpiadidelleNeuroscienze", Turin, Italy (09/03/2024)
● Editorial duties:	
● others	Posters at meetings: Chicote J, Mezzanotte M, Scimia N, Toce A, Stanga S. "Iron and βamyloid deposition pattern in the adult 5xFAD mouse model of Alzheimer's disease." I Congresso del comitato giovani degli Amici della Morfologia "MorFuture" Turin, Italy (12-13/04/2024) Chicote J, Mezzanotte M, Vergara C. Toce A, Rosano V, Rodríguez-Pascau L, Pizcueta P, S. Stanga. "New mechanisms and strategies to prevent AD." XXXIV Congresso Nazionale Gruppo italiano per lo studio della neuromorfologia (GISN) Catania, Italy (22-23/11/2024)
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Otherorganizational activities:	na
Workshops, Schools or Conferences organized:	Member of the local organizing committee at Workshop "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies" (III edition), Torino, Italy (08-09/11/2024)
Technology transfer achievements (patents, etc.):	

Giulia Iezzi, Research fellow

Supervised PhD students:	
Honors, prizes, awards:	na
Outreach activities	
● International collaborations:	na
● Invited talks:	na
● Science communication:	na
● Editorial duties:	na
● Others	Poster at meeting: Iezzi G , Schellino R, Caretto A, Boido M, Vercelli A. “Lack of smn protein significantly affects the survival and morphology of population of projection neurons in the sensorimotor cortex of a murine model of spinal muscular atrophy”. Motor NeuronDiseases, Turin, Italy (08–09/11/2024)
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Otherorganizational activities:	na
Workshops, Schools or Conferences organized:	Member of the local organizing committee at Workshop "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies” (III edition), Turin, Italy (08–09/11/2024)
Technology transfer achievements (patents, etc.):	na

Alessandra Tuninetti, Research fellow

Supervised PhD students:	
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
Otherorganizational activities:	na
Workshops, Schools or Conferences organized:	Member of the local organizing committee at Workshop “Motor neuron diseases: understanding the pathogenetic mechanisms to develop therapies” (III edition), Torino, Italy (08-09/11/2024)
Technology transfer achievements (patents, etc.):	

Anna Caretto, PhD Student

Supervised PhD students:	na
Honors, prizes, awards:	DMM Conference Travel Grant for the Neuroscience Conference 2024 (Chicago, USA, 05-09/10/2024)

Outreach activities	
● International collaborations:	Prof. Alain Prochiantz (Paris, France)
● Invited talks:	na
● Science communication:	Member of the local organizing committee at "OlimpiadidelleNeuroscienze", Turin, Italy (09/03/2024) Member of the local committee for "Pint of Science", Turin, Italy (14/05/2024) Member of the organizing committee for the stand "Esplorando la menteumana" in the LaboratoridellaRicerca Area for the U*NIGHT event, Turin, Italy (28/09/2024)
● Editorial duties:	na
● others	Posters at meetings: Caretto A, Vargas-Abonce SE, Prochiantz A, Di Cunto F, Boido M, Vercelli A. Analysis of glycinergic system alterations in SpinalMuscularAtrophy. 4th International Scientific Congress on Spinal Muscular Atrophy (SMA Europe 2024). Ghent, Belgium (14-16/03/2024) Caretto A, Di Cunto F, Vargas-Abonce SE, Prochiantz A, Boido M, Vercelli A. Study of possible glycinergic system alterations in Spinal Muscular Atrophy. "MorFuture: gli studi di morfologia tra tradizione e innovazione". Turin, Italy (12-13/04/2024) Caretto A, Di Cunto F, Vargas-Abonce SE, Prochiantz A, Boido M, Vercelli A. Unravelling the contribution of mitochondrial SMN1-anticorrelated genes in determining early symptomatic glycinergic system alterations in Spinal Muscular Atrophy. Neuroscience 2024 (SfN), Chicago, USA (05-09/10/2024) Caretto A, Di Cunto F, Vargas-Abonce SE, Prochiantz A, Boido M, Vercelli A. Unravelling the contribution of mitochondrial SMN1-anticorrelated genes in determining early symptomatic glycinergic system alterations in a Spinal Muscular Atrophy murine model. Workshop "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies" (III edition), Turin, Italy (08–09/11/2024) Caretto A, Di Cunto F, Vargas-Abonce SE, Prochiantz A, Boido M, Vercelli A. Unravelling the contribution of mitochondrial SMN1-anticorrelated genes in determining early symptomatic glycinergic system alterations in a mouse model of Spinal Muscular Atrophy. SINS National meeting of PhD Students in Neuroscience, Naples, Italy (12/11/2024)
Organizational activities and responsibilities at NICO:	Responsible for the electrophoresis room
Speakers invited:	na
Otherorganizational activities:	na
Workshops, Schools or Conferences organized:	Organization of the First Congress of the "Amici dellaMorfologia" young Committee "MorFuture: glistudi di morfologiatratradizione e innovazione", Turin, Italy (12-13/04/2024) Member of the local organizing committee at Workshop "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies" (III edition), Turin, Italy (08–09/11/2024)

	Organization of the SINS "PhD Student National Meeting in Neuroscience", Naples, Italy (12/11/2024)
Technology transfer achievements (patents, etc.):	na

Chiappini Vanessa, PhD student

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
● International collaborations:	ChristelGenoud (UNIL, Lausanne, Switzerland)
● Invited talks:	"Three-dimensional insights into neuro-glia interaction: the role of SBF-SEM", Nanoinnovation (Rome), September 12th 2024. "New Frontiers in Volume Electron Microscopy and its application in Neuro-Glia interaction", 3D Volume Microscopy beyond CLEM (IFOM, Milan), October 15th 2024.
● Science communication:	
● Editorial duties:	
● Others	Posters at meetings: Morphological alterations in Lafora disease: an ultrastructural analysis using SBF-SEM, PhD meeting SINS, Napoli, 13/11/2024.
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Otherorganizational activities:	na
Workshops, Schools or Conferences organized:	Member of the local organizing committee at Workshop "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies" (III edition), Turin, Italy (08–09/11/2024)
Technology transfer achievements (patents, etc.):	

Sveva Dallere, PhD student

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
● International collaborations:	J. Willem Vrijbloed (PharmafoxTherapeutics AG); R. Fariello (PharmafoxTherapeutics AG); Makoto Sato and YuichiroOka (Osaka University, Japan)
● Invited talks:	na
● Science communication:	na
● Editorial duties:	na
● others	Oralcommunication: Dallere S, Boido M, Vercelli A. "Terpenes and Alzheimer's Disease: a preliminary in vitro study" IBRO workshop on "Climate Change Effect on CNS Re-Programming and Associated Disorders" Online (02/01/2024) Posters at meetings:

	Dallere S, Chasseur S, Boido M, Vercelli A. "Terpenes and Alzheimer's disease: a study in stem cell derived models" MorFuture Congress, Turin, Italy (12-13/04/2024) Dallere S, Chasseur S, Boido M, Vercelli A. "Terpenes and Alzheimer's disease: an in vitro study" FENS Forum 2024 Wien, Austria (25-29/06/2024) Dallere S, Schellino R, Vrijbloed W, Pacca P, Fariello R, Boido M, Vercelli A. "ActR-Fc-nLG3: a new molecule that supports neuromuscular junction innervation and enhances motor endurance and strength", Workshop "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies" (III edition), Turin, Italy (08–09/11/2024) Dallere S, Chasseur S, Pavarino M, Sgorbini B, Boido M, Vercelli A. "Investigating the effects of terpenes on Alzheimer's Disease: an in vitro research", SINS National meeting of PhD Students in Neuroscience, Naples, Italy (12/11/2024)
Organizational activities and responsibilities at NICO:	Member of the Green Policy Committee
Speakers invited:	na
Otherorganizational activities:	na
Workshops, Schools or Conferences organized:	Member of the local organizing committee at Workshop "Motor Neuron Disease: understanding the pathogenetic mechanism to develop therapies" (III edition), Turin, Italy (08-09/11/2024)
Technology transfer achievements (patents, etc.):	na

Sofia Dotta, PhD student

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
● International collaborations:	Prof. Michaela Kress (Medical University Innsbruck), Dr. Franziska Rother (MBC, Berlin)
● Invited talks:	Dotta S, Musso G, Testa L, Parmar A, Bader M, Rother F, Marvaldi L. Title: Importin alpha3 controls age-dependent axonal branching by modulating protein expression. 7th International Symposium on PeripheralNerveRegeneration. Milan, Italy (07-09/05/2024)
● Science communication:	na
● Editorial duties:	na
● others	Poster at meeting: Christian O. Pritz, Giada Musso, Sofia Dotta, Franziska Rother, Michael Bader, NataliyaOkladnikov and Letizia Marvaldi. "Quantifying paw motor function and posture after sciatic nerve injury keypoint segmentation" at "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies" (III edition), Turin, Italy (08-09/11/2024)
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Otherorganizational activities:	Member of the local organizing committee at workshop "Motor

	Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies" (III edition), Turin, Italy (08-09/11/2024)
Workshops, Schools or Conferences organized:	na
Technology transfer achievements (patents, etc.):	na

Clelia Ferrero, 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 "OlimpiadidelleNeuroscienze", Turin, Italy (09/03/2024)
● Editorial duties:	na
● others	Posters at meetings: Ferrero C. et al. "IntracerebroventricularhNSCgraft in ALS mice: first pre-clinical results", "MorFuture: gli studi di morfologia tra tradizione e innovazione", Turin, Italy (12-13/04/2024) Ferrero C, Signorino E, Vercelli A, Boido M. "Modeling Frontotemporal Dementia (FTD) pathogenesis with human cerebral organoids" ; Unlocking human brain complexity using 3D culture and single-cell omics, Capri, Italy (13-16/10/2024) and Workshop "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies" (III edition), Turin, Italy (08–09/11/2024) Ferrero C, Signorino E, Vercelli A, Boido M. "Developing human brain models to investigate the origins of frontotemporal dementia (FTD)", SINS National meeting of PhD Students in Neuroscience, Naples, Italy (12/11/2024)
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Otherorganizational activities:	na
Workshops, Schools or Conferences organized:	Member of the local organizing committee at Workshop "Motor Neuron Disease: understanding the pathogenetic mechanism to develop therapies" (III edition), Turin, Italy (08-09/11/2024)
Technology transfer achievements (patents, etc.):	na

Ersilia Nicorvo, PhD Student

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
● International collaborations:	Piera Smeriglio (Paris, France)
● Invited talks:	

● Science communication:	Member of the local organizing committee at "OlimpiadidelleNeuroscienze", Turin, Italy (09/03/2024)
● Editorial duties:	
● Others	Posters at meetings: Nicorvo E, Rasà DM, Stanga S, Santonicola P, Matino I, Montefusco S, Medina D, Vercelli A, Smeriglio P, Di Schiavi E, Boido M. "Identification of new SMN-independentcompounds to counteractSpinalMuscularAtrophy: in vitro validation", SMA Europe 4th International Scientific Congress on SpinalMuscularAtrophy, Ghent, Belgium (14-16/03/2024) Nicorvo E, Rasà D M, Stanga S, Santonicola P, Matino I, Montefusco S, Medina D, Vercelli A, Smeriglio P, Di Schiavi E, Boido M. "Identification of new SMN-independentcompounds to counteractSpinalMuscularAtrophy: in vitro validation", Congresso MorFuture: gli studi di morfologia tra tradizione e innovazione, Turin, Italy (12-13/04/2024) Nicorvo E, Rasà DM, Stanga S, Santonicola P, Matino I, Montefusco S, Medina D, Vercelli A, Smeriglio P, Di Schiavi E, Boido M. "Discovery and in vitro validation of novel SMN-independent compounds to combat Spinal Muscular Atrophy", Workshop "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies" (III edition), Turin, Italy (08–09/11/2024) Nicorvo E, Ferraro A, Boido M, Di Cunto F, Vercelli A. "Exploring neuron-tumor crosstalk using in vitro models: co-culture systems and morphological analysis", SINS National meeting of PhD Students in Neuroscience, Naples, Italy (12/11/2024)
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Otherorganizational activities:	na
Workshops, Schools or Conferences organized:	Member of the local organizing committee at Workshop "Motor Neuron Disease: understanding the pathogenetic mechanism to develop therapies" (III edition), Turin, Italy (08-09/11/2024)
Technology transfer achievements (patents, etc.):	

Gianna Pavarino, PhD student

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
● International collaborations:	na
● Invited talks:	na
● Science communication:	Poster presenterat the dissemination event "U*NIGHT - La Notte Europea delle Ricercatrici e dei Ricercatori 2024 - Caffe Scientifico, The Green Brain. "Il cervello in un bosco: vivere il verde come terapia non farmacologica e per prevenire le malattie neuropsichiatriche", Turin, Italy (28/09/2024)
● Editorial duties:	na

• others	Posters at meetings: Pavarino G, Brasso C, Schellino R, Carluccio A, Cirulli F, Boido M, Rocca P, Vercelli A. "The biologicaleffects of "green-therapy" on MDD". MorFuture: gli studi di morfologia tra tradizione e innovazione, Turin, Italy (12-13/04/2024) Pavarino G, Brasso C, Schellino R, Carluccio A, Cirulli F, Boido M, Rocca P, Vercelli A. "The biologicaleffects of "green-therapy" on MDD". FENS Forum 2024, Vienna, Austria (25-29/06/2024) Pavarino G, Brasso C, Schellino R, Carluccio A, Cirulli F, Boido M, Rocca P, Vercelli A. "The biologicaleffects of "green-therapy" on Major Depressive Disorder" Neuroscience 2024, Chicago, USA (05-09/10/2024) Pavarino G, Brasso C, Schellino R, Carluccio A, Cirulli F, Boido M, Rocca P, Vercelli A. "The biological effects of "green-therapy" on MDD patients" National meeting of PhD students in Neuroscience 2024, Naples, Italy (12/11/2024)
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Otherorganizational activities:	na
Workshops, Schools or Conferences organized:	Member of the local organizing committee at Workshop "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies" (III edition), Turin, Italy (08-09/11/2024)
Technology transfer achievements (patents, etc.):	na

Daniela Maria Rasà, PhD student

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
• International collaborations:	Dr. Martinat (I-STEM, Corbeil-Essonnes, France); EdorKabashi, Institut Imagine (Paris, France)
• Invited talks:	na
• Science communication:	na
• Editorial duties:	na
• others	Posters at meetings: Rasà DM, Stoppa I, Boido M. "The investigation of the stress effects on amyotrophiclateralsclerosisonset and/or progression in an in vitro disease-predisposedcondition", MorFuture: gli studi di morfologia tra tradizione e innovazione; Turin, Italy (12-13/04/2024) Rasà DM, Stoppa I, Berenger-Currias N, Ciura S, Kabashi E, Martinat C, Boido M. "study of stress impact on amyotrophic lateral sclerosis pathogenesis", Workshop "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies" (III edition), Turin, Italy (08–09/11/2024) Rasà DM, Stoppa I, Berenger-Currias N, Ciura S, Kabashi E, Martinat C, Boido M. "the stress impact on amyotrophic lateral sclerosis", XXXIV congresso GISN 2024, Catania, Italy (22-

	23/11/2024)
Organizational activities and responsibilities at NICO:	Member of the Green Policy Committee
Speakers invited:	na
Otherorganizational activities:	na
Workshops, Schools or Conferences organized:	Member of the local organizing committee at Workshop “Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies” (III edition), Turin, Italy (08-09/11/2024)
Technology transfer achievements (patents, etc.):	na

Noemi Scimia, PhD student

Supervised PhD students:	na
Honors, prizes, awards:	na
Outreach activities	
● International collaborations:	MinoryxTherapeutics (Spain, Belgium)
● Invited talks:	na
● Science communication:	na
● Editorial duties:	na
● others	na
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Otherorganizational activities:	na
Workshops, Schools or Conferences organized:	Member of the local organizing committee at Workshop "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies” (III edition), Torino, Italy (08-09/11/2024)
Technology transfer achievements (patents, etc.):	na

Veloz-Castillo, Maria Fernanda, PhD student

Supervised PhD students:	na
Honors, prizes, awards:	Dean's Travel Award 2024
Outreach activities	
● International collaborations:	ChristelGenoud (UNIL, Lausanne, Switzerland)
● Invited talks:	
● Science communication:	Representation of Mexican Women in Science in Wikipedia (October 2024) Wiki-Conference North America, University of Indianapolis, Indianapolis, USA Indiana’s Crossroads: Celebrating BIPOC Pioneers of the Americas (October 2024)
● Editorial duties:	na
● Others	Posters at meetings: “Spatial distribution of glycogen in layer 1 of the somatosensory cortex in aging mice”, Connectomics Conference, Berlin, Germany (June 2024) “Optimization of anesthesia and microwave fixation conditions

	for biochemical and morphological studies of glycogen metabolism after behavioral studies" Neuroscience 2024, Chicago, USA (05-09/10/2024)
Organizational activities and responsibilities at NICO:	na
Speakers invited:	na
Otherorganizational activities:	Organization of "edit-a-thons", events where I teach people how to edit Wikipedia and create new articles related with science and scientists. Women in science, Online event (February 2024); Organization of a photography campaign to promote women in science across the Wikimedia projects (February 2024).
Workshops, Schools or Conferences organized:	Member of the local organizing committee at Workshop "Motor Neuron Diseases: understanding the pathogenetic mechanisms to develop therapies" (III edition), Torino, Italy (08-09/11/2024)
Technology transfer achievements (patents, etc.):	na

4. Research activity in 2024

a. Summary

We study CNS/PNS development and the neurobiological mechanisms and molecular pathways leading to normal development, neurodegeneration and pain. 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

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 networks 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, identify early disease-related alterations, 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 and exploit correlative microscopy approaches, 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 in this new era.

c. Objectives

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

Astrocytes-neuronsstructuralcrosstalk
CC acquired a new dataset from brain fixated with a high-power microwave setup in KAUST (Saudi Arabia). This technique better preserves the astrocytic glycogen pool and allows to detect levels 3x higher than normal perfusion.
A Metaverse space application has been developed, used to perform collaborative structural analysis of the vEM datasets.

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 together with morphological alterations of these pyramidal cell types, ii) an association between SMN deficiency and neuronal dysregulation of GABAergic signaling and metabolism.
MB is also testing different therapeutic approaches. We demonstrated that the ICV injection of EVs isolated from adipose-derived MSCs had positive effects on SMA progression of SMA mice (Virla et al., 2024). 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, in different SMA models.
Stem cell therapy in ALS
MB and AV (in coll. with D. Ferrari; with DMR and CF) optimized a non-clinical protocol of ICV human neural stem cell transplantation in $SOD1^{G93A}$ mice and demonstrated the safety and efficacy.
Spinal cord injury
MB (in coll. with C. Tonda-Turo, with SC) is developing a 3D in vitro spinal cord model by bioprinting technique. Different cell types were bioprinted with above aligned fibers, improving neuronal differentiation. The cells showed proper cellular differentiation and functional connectivity. Preliminary injury tests have been conducted.
Lifestyle & Active and Healthy Ageing

AV, MB, RS (with GP) are investigating the effect of terpene exposure on depressed patients: after 6 weeks of treatment, the depressive symptoms were improved, together with lower levels of inflammatory biomarker IL6, and higher concentrations of adiponectin (paper submitted). Whit SD, we also observed positive effects of green exposure treating *in vitro* both murine and hiPSC-derived AD neurons.
In coll. with Pharmafox, AV, MB, RS (with SD and PP) are studying the MoA of ActR-Fc-nLG3 in vitro.
MB&DMR are evaluating how stress can affect ALS pathogenesis in disease-predisposed conditions: we identified a crucial role of the PI3K/Akt pathway in mediating stress response in different experimental ALS-predisposed conditions.
SS (with MM, JCG and NS) are studying the effects of long-term regular running exercise in mice during healthy aging, to verify its beneficial effects on brain iron overload, trafficking and consequently on improving cognitive abilities.

Aging and Alzheimer's disease (AD)
SS (with MM, JCG and NS) are evaluating the iron levels available for intracellular metabolic reactions in the brain, their implications in determining cytotoxic effects, the decline in cognitive/motor skills and how mitochondria, iron and amyloid deposits interact in the brain.
In collaboration with Marini and Bertinaria (Univ. Torino) and Minoryx Therapeutics, they are testing different molecules to identify new potential therapies for AD.

Investigation of Importin alpha3 (IMPA3) mutant mice in neuronal regeneration, sex and ageing
In coll. with F. Rother (MDC, Berlin, Germany), LM (with SDo) 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. In coll. with M. Fainzilber and I. Rishal (Weizmann Inst. Science, Israel) LM is completing two projects: i) to study the role of KLF6 TF during development, ii) to study the paw posture, motor function and pain (with C.O. Pritz and SDo).

e. Advancement in the field

Our group works on 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 2024, we have obtained significant results in the field of SMA and MN diseases, by identifying new therapeutic approaches, which extend the lifespan of the animal models of disease, improve motor performance and delay MN neurodegeneration.
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, with a correlative microscopy approach.

f. Publications

Audouard E, Khefif N, Gillet-Legrand B, Nobilleau F, Bouazizi O, Stanga S, Despres G, Alves S, Lamazière A, Cartier N, Piguet F. 2024. Modulation of Brain Cholesterol Metabolism through CYP46A1 Overexpression for Rett Syndrome. Pharmaceutics, Jun 3;16(6):756.
Research article - Q1

Calì C. 2024. Regulated exocytosis from astrocytes: a matter of vesicles? Frontiers in Neuroscience 18, 1393165
Opinion article - Q2

Calì C, Agus M. 2024. NeuroVerse: Immersive exploration of 3D ultrastructural brain reconstructions for education and collaborative analysis. Proceedings of the 29th International ACM Conference on 3D Web Technology, 1-10.
Conference Proceeding - n.a.

Calì C, Cantando I, Veloz Castillo MF, Gonzalez L, Bezzi P. 2024. Metabolic Reprogramming of Astrocytes in Pathological Conditions: Implications for Neurodegenerative Diseases. International Journal of Molecular Sciences 25 (16), 8922
Review - Q1

JH Kam, M Billeres, L Herault, C Cali, B Sarmiento, P Cassano, Magistretti P, Mitrofanis J. 2024. Exploring current and future technologies to make sense of the biophoton phenomenon: a narrative review. Advanced Technology in Neuroscience 1 (2), 201-210.
Review - n.a.

Lembo D, Abate Daga F, Calì C, Garbossa D, Manfredi M, Odetto L, Ostacoli L, Paccotti P, Raimondo S, Reimondo G, Sciascia S. 2024. Early introduction of simulation in the medical curriculum: the MedInTo perspective. Frontiers in Medicine 10, 1280592.
Community Case Study - Q1

Mezzanotte M, Stanga S. 2024 Brain Iron Dyshomeostasis and Ferroptosis in Alzheimer's Disease Pathophysiology: Two Faces of the Same Coin. Aging and disease. Jun 14; Online ahead of print.
Review - Q1

Musso G, Dotta S, Parmar A, Rasà DM, Di Cunto F, Marvaldi L. 2024 Standardization of a Novel Semi-Automatic Software for Neurite Outgrowth Measurement. J Vis Exp. Aug 9;(210).
Research article - Q3

Schellino R, Boido M, Vrijbloed JW, Fariello RG, Vercelli A. 2024 Synergistically Acting on Myostatin and Agrin Pathways Increases Neuromuscular Junction Stability and Endurance in Old Mice. Aging Dis. Apr 1;15(2):893-910. doi: 10.14336/AD.2023.0713-1.
Research article - Q1

Testa L, Dotta S, Vercelli A, Marvaldi L. 2024 Communicating pain: emerging axonal signaling in peripheral neuropathic pain. Front Neuroanat. Jul 9;18:1398400.
Review - Q1

Vercelli A, Boido M. Giuseppe Levi (1872-1965) intellettuale e neuroscienziato tra le due guerre. 2024. "Grandi personaggi della medicina italiana nei secoli", Padova 2023.
Review - n.a.

Virla F, Turano E, Scambi I, Schiaffino L, Boido M*, Mariotti R*. 2024. Administration of adipose-derived stem cells extracellular vesicles in a murine model of spinal muscular atrophy: effects of a new potential therapeutic strategy. Stem Cell Res Ther. Apr 1;15(1):94.
Research article - Q1

5. Future directions and objectives for next years

a. Summary

We will exploit our previous research on i) axonal growth in the CNS and PNS, 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 AD). By investigating the role of mitochondrial-iron metabolism and physical exercise in healthy aging and AD, we also aim to understand why elderly people present systemic anemia but accumulate iron in the CNS, a feature 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: 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. In the PNRR Mnesys project, we are also creating biological models in vitro from iPSCs and organoids from patients, to identify new markers of disease (AD). These projects are 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, correlative microscopy, 3D EM, and semi-automated tool for 3D analysis in VR.

b. Background and Significance

There is a growing interest in studying the development and disease of the CNS in terms of networks: genes, miRNAs and molecular networks at a ultramicroscopic level of magnitude, synaptic networks at the microscale, and anatomical and functional networks at the meso- and macroscale. Perturbances in the networks at the different scale levels may result in developmental or neurodegenerative disorders. 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 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.
More recently, AV has been awarded a grant to apply Robotics in elderly care. The project (Daisy&Ron) is coordinated by him and is in collaboration with Teoresis and Intravides.

c. General aim and integration with mission of the Institute

Our research aims to understand some basic mechanisms of neural development, whose alterations may be involved in the onset of neuropsychiatric diseases and of 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 and pain state. 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

Astrocyte-neuron interplay
Thanks to the new vEM Zeiss Volutome, we are now able to improve the resolution of our observation to the nm scale. Thanks to a collaboration with R. Alkhateer, expert in Lafora Disease, we are acquiring high-resolution dataset from a Laforin-KO genetic model, to investigate how the structural relationship between the Lafora bodies’ accumulations and the synapses in the CA1 contribute to the onset of the disease.

Cortical development
In collaboration with prof Makoto and Oka (Osaka, Japan), AV, together with Sveva Dellere and the help of the microscopy technician is studying pyramidal neuron development. Moreover, together with prof Pizzagalli is studying the development of sulci and gyri in cerebral cortex, particularly that of Rolandic Scissure, in large human dataset, correlated with neurodevelopmental diseases.

Altered cellular and molecular mechanisms in motor neuron diseases and therapeutic approaches
SMA: MB, AV, RS (with AC, GI and FLR) are studying the morphological and numerical alterations of projection neurons in SMA cortical cytoarchitecture, 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 the studies on SMN influence on neurotransmitter metabolism and release among neurons and glia in cortex and spinal cord.
With AC and FLR (in coll. with Dr Schieweck and Viero), we will study the SMN role in regulating methylated RNAs in neurons, to elucidate its impact on the epitranscriptome and translatome.

Moreover, MB (with GM, ES, DMR, SS) will further test both SMN-dependent and -independent approaches for SMA, testing in vivo repurposed drugs, also in combination with the available SMN-dep therapies.
SMA/ALS: SS (with MM, JCG and NS) will study mitochondrial mobility and function by targeting mitochondrial enzymes and kinases in hiPSC-MNs, in collab. with V. Valsecchi, C. Svendsen and A. Prochiantz.

Spinal cord injury
MB (and SC) is developing a 3D in vitro spinal cord model by bioprinting technique. We will include a third murine cell line, testing its compatibility in the co-culture, together with molecules to improve axonal growth. In parallel, a new model including human cells will be developed.
AV and PP are actually collaborating to study the glymphatic system (the brain washing machine") in SCI and neurodegenerative diseases.

Aging and Alzheimer's disease (AD)
SS (with MM, JCG and NS) by using cellular and animal models of healthy aging and AD will evaluate new possible mechanisms implicated in the cognitive and motor decline in AD and the possible mechanism behind the beneficial effect of long-term regular exercise during healthy aging.
AV, MB, RS and PP (with GM and SDe), in coll. with Pharmafox Therapeutics AG, will further test molecules for supporting muscle innervation in elderly, possibly as combinatorial treatments.

Mental health and urban green
In coll. with Prof. Rocca (UniTo) and F. Cirulli (ISS), AV, MB, RS (and AT, SD) will continue studying the effects of living in the green on depression, schizophrenia and AD, from a clinical, behavioral and biochemical marker point of view. This will be a preliminary study in order to prepare the group for the new Green deal program of Horizon Europe.

Neuropathic pain
AV, LM, SDo 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, SC, AM) are exploiting iPSCs and organoids to identify early cellular and ultrastructural changes/alterations, as possible predictive biomarkers. With EN, we are also exploring *in vitro* the neuron-tumor interactions. AV, LM and SDo in coll. with A. Buffo and G. Gambarotta will implement iPSCs sensory neurons to identify therapeutic target markers.

e. Unique features of the project research

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**

The collaboration with the Polytechnic and INRIM (Istituto Nazionale di RicercaMetrologica) will allow to design biosensors and lab-on-chip for 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 neuronal tracers 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 at different postnatal ages (MB, RS together with AC, GI and FLR), 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. The installation of a new super-resolution confocal microscope is planned in 2025. All these techniques will also 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 2024

Laboratory name: **Clinical Neurobiology**

1. LABORATORY DESCRIPTION – PERSONNEL:

Principal Investigator

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) Martire, Serena (until 05/2024)
   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

3) 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

4) 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

5) Mohamed Abdel Azim, Gada (until 27/11/2024)
   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

6) 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 AmountFunded
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

3. SCIENTIFIC ACTIVITIES IN 2024

Alessia Di Sapio (acting PI)

Supervised PhD students:	NA
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	
• Invited talks:	Space of Care as one for patients 4-5 aprile 2024, Milano PLUSM: efficacia, maneggevolezza e personalizzazione della terapia in SM, Roma 10-11 maggio 2024 Lecture presso Master Universitario"Mind&Move Health Specialist – Specialista della Salute Psico-Fisica", Torino 12 luglio 2024 Il Tempo conta: condivisione di cluster di pazienti per tempestiva presa in carico dei pazienti con amiloidosi, Orbassano 6 novembre 2024 Perspective in MS nuove prospettive di cura per il paziente con SM, Verona 5-6 dicembre 2024 Biomarking the future in MS: gli NFLin pratica clinica, benefici in evidenza, Milano 11 dicembre 2024
• 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.):	

Arianna Sala, Resident Biologist

Supervised PhD students:	NA
Honors, prizes, awards:	
Outreach activities	
• International collaborations:	
• Invited talks:	MS Dimension: evoluzione e nuove opportunità in Sclerosi Multipla. Lancio Italiano del biosimilare di Natalizumab. Intervento dal titolo:Il test JCV e l'offerta di Sandoz in MS. Roma 14-15 Maggio 2024, Villa Pamphili
• Science communication:	
• Editorial duties:	Report scientifico sul tema: Neurodegenerazione e Progressione di malattia nella SM. 40th ECTRIMS Meeting, Copenhagen, September 17-20, 2024.
• others	
Organizational activities and responsibilities at NICO:	
Speakers invited:	
Other organizational activities:	
Workshops, Schools or Conferences organized:	
Technology transfer achievements (patents, etc.):	

Serena Martire, Biostatistician

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

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:	"NFL: focus sul test journey" (editorial project with Novartis)
• 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) Winner of FISM Fellowship 2023, starting from March 2024; title of the project "Implementation of GFAP, in addition to NFL, in progressive multiple sclerosis patients: a potential tool for objective measuring of clinical and patient reported outcomes" Presenting author for poster titled *"Integrability of the new International MOGAD Panel Proposed Criteria for MOG-IgG detection in a real-world diagnostic setting using a live cellbased flow cytometry assay"* 40th ECTRIMS Meeting, Copenhagen, September 17-20, 2024. Presenterduring an online webinar at Biotech Week 2024 titled "Le Biobanche A Supporto Della Ricerca E Della Medicina Personalizzata"
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	Presenting author for poster titled *"sNFL as additional biomarker for clinical management of multiple sclerosis patients at relapse"* at the40th ECTRIMS Meeting,

	Copenhagen, September 17-20, 2024.
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:	
• 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.):	

4. Research activity in 2024

a. Summary

In the field of multiple sclerosis, in particular in the context of progressive forms of the disease, there is an increasing need to implement cost-effective biological fluid markers, to evaluate their role as additional tests to predict and measure progression and therapy effectiveness. In this context, we are exploring the role of serum glial fibrillary acidic protein (GFAP, marker of astrocytic damage) and serum neurofilament light-chain (NFL, marker of neurodegeneration and neuroinflammation) as possible candidates for patients' monitoring.

b. Background and rationale

To promptly detect disease progression and evaluate therapy effectiveness, as well as aid in everyday clinical management, studies have been carried out to identify monitoring biomarkers, among which glial fibrillary acidic protein (GFAP) and neurofilament light-chain (NFL). Specifically, NFL is considered an optimal biomarker for neurodegeneration, inflammation and therapy effectiveness; GFAP is a marker of astrocytic damage and is currently being studied as a promising biomarker of ongoing disease progression.
GFAP is a type-III intermediate filament of mature astrocytes, in both central and peripheral nervous system, which purpose is to preserve synapses' normal metabolic function and to concur to BEE integrity. Several studies on GFAP have highlighted its possible role in in-vivo longitudinal evaluation of the astrocytic response in several neurological disorders. At first, researches have been carried out on cerebrospinal fluid (CSF), where GFAP levels are 100 times higher than in blood and dosing is possible with traditional immunoassays. More recently, the development of extremely sensitive and specific techniques, such as single molecule arrays (Simoa) has enabled GFAP quantification both in healthy individuals and patients serum, allowing studies on GFAP implementation in everyday clinical practice.
GFAP is generally higher in PMS patients, compared to healthy controls and RRMS patients (active and in remission), but lower compared to patients suffering from neuromyelitis optica spectrum disorders (NMOSD).
Despite some conflicting data, GFAP levels have been seen to correlate with disease duration and severity (EDSS), MRI lesion load and other neurodegeneration biomarkers like serum neurofilament light-chain (NFL). Still, GFAP relationships with RAW (relapse-associated worsening), PIRA (progression independent from relapse activity) and future disability accrual have to be further explored.
Some more issues are still to be assessed to implement serum GFAP in the clinical practice. Since GFAP is known to increase with physiological aging and depend on other confounding factors, its levels are still to be properly assessed to elaborate definite and common decade cut-offs. On the matter, Tybirk et al. recently elaborated age-dependent reference values on serum of a healthy cohort.
In MS symptoms as fatigue and cognitive impairment are of utmost importance and often the first signs of progression; nevertheless, they can be challenging to detect and measure in clinical practice
Data about GFAP levels' correlation with clinical parameters as patient reported symptoms and outcomes (PROs) and cognitive functions are still lacking in multiple sclerosis casuistry, while have been already found in other pathologies, like COVID-19 and Alzheimer disease .

c. Objectives

To fill the above-mentioned gaps in knowledge, this retrospective study aims to:
1. Define GFAP reference values in healthy controls (HC);
2. Clinically and biologically (GFAP and NFL) monitor PMS (progressive MS) patients' progression during the disease course;
3. Early identify the transitional phase from RRMS to SPMS, when progression, already present as biological phenomena, reaches the clinical threshold.

Both biomarkers will be tested to understand their ability as objective additional (and possible future surrogate)
monitoring biomarkers of various aspects of progression in the field of PMS.
The combination of NFL (as marker of axonal damage) and GFAP (as marker of astrocytic damage) will allow to monitor inflammatory state (NFL) and disability accumulation (GFAP), as well as to go into details of disease components throughout follow-up, thanks to the differential biomarker characteristics.

d. Results

Objective 1. Definition of GFAP reference values on healthy subjects

Technical performance

Simoa Neuro 2-plex B Advantage kit (Quanterix, CA) was used on our SR-X instrument to test 139 healthy subjects (not having neuroimmunological disorders and not being a first-degree relative to a person with MS), who signed the informed consent for the CRESM Biobank and donated a blood sample.
A total of 7 plates were used, with a mean R2 = 0.989 (min R2 = 0.968; max R2 = 0.998) for NFL and mean R2 = 0.975 (min R2 = 0.953; max R2 = 0.997) for GFAP calibration curves. Subjects' samples were tested in duplicate.
Controls obtained by pooling 7 samples having known low NFL values showed a mean inter-assay CV of 25% for both NFL and GFAP.
Additionally, three samples out of the 139 were chosen (respectively low, medium, high GFAP levels) to be repeated in duplicate along five plates to asses intra- and inter-variability of the test. Results are the following:
- NFL: mean intra-assay variability: 12%, mean inter-assay variability: 6.5%;
- GFAP: mean intra-assay variability: 15%, mean inter-assay variability: 16%.

Cut-off determination
GFAP demonstrated a not-normal distribution (Shapiro-Wilk test p-value<0.001), right skewness (1.713) and high kurtosis (7.783). On the other hand, log-transformed GFAP showed a normal distribution (Shapiro-Wilk test p-value=0.665).
Pearson correlation was used to assess the relationship between log-GFAP and age, BMI and BV. GFAP was seen to increase with age (cor = 0.440; p-value<0.001; R2=0.193), with an annual increment of 2.8%. Moreover, the analysis revealed a significant negative correlation with BV (cor = -0.297; p-value=0.005; R2=0.088).
Welch two sample t-test was used to assess differences in log-GFAP between sexes: we found a statistical difference between groups, showing lower levels of log-GFAP in males compared to females (t = -2.149; p-value=0.036). Mann Whitney U test was used to confirm the difference in sex for GFAP, however being poorly evident (p-value = 0.0484).
Lastly, Kruskal Wallis rank-sum test with Dunn post-hoc test was used to assess differences between decades. GFAP increased significantly in the 5th decade (median = 103.62 pg/ml, range 49.47 – 260.45) compared to both the 2nd (median = 72.07 pg/ml, range 35.80 - 155.30; p= 0.002) and the 3rd decade (median = 72.70 pg/ml, range 20.17 – 165.86 ; p= 0.002). Same results were seen in the 6th decade (median = 114.82 pg/ml, range 45.38 – 289.37) compared to the 2nd (p<0.001) and 3rd decade (p<0.001).
To obtain normative values, we decided to focus on the relationship between GFAP and age (explaining 20% of log-GFAP variation), in order to not over-complicate the model and possibly cause overfitting. Several models were tried and evaluated with parameters such as residual plotting, QQplot, variance, bias, errors and coverage probability. Overall, in terms of interpretability and clinical use, GCRQ for decades looks like the best option. Obtained normative values (with 95%CI) are the following:

DECADES (years)	50TH – pg/ml [95CI]	75TH – pg/ml [95CI]	90TH – pg/ml [95CI]
20-39	74.25 [62.23 – 88.70]	92.90 [78.59 – 109.83]	121.32 [102.06 – 144.52]
40-49	84.37 [70.50 – 100.99]	105.00 [89.57 – 123.67]	138.11 [116.73 – 163.05]
50-59	96.80 [77.48 – 117.93]	118.39 [98.44 – 142.41]	157.56 [128.89 – 191.70]
60-70	112.14 [87.15 – 143.74]	138.81 [109.92 – 175.48]	188.54 [143.06 – 250.22]

Objective 2. Clinically and biologically (GFAP and NFL) monitoring of primary progressive patients (PPMS)

At Regional referral MS Center a series of PMS patients undergo to longitudinal multidimensional evaluations (indicatively once every six months, every 12 months at most) including physical and cognitive performances and PROs collection, by personnel recruited thanks to funds of " Bando Roche per iServizi" Together with such assessments, patients are being followed-up with radiological and clinical assessments and are regularly enrolled (every six months) in blood draws for CRESM Biobank, guaranteeing a repository of high-quality samples.
The collected serum samples are tested for GFAP and NFL levels. Plus, we are performing data analysis, evaluating the correlation of NFL and GFAP measurement with functional tests and PROs, as well as with other clinical parameters, correcting for clinical and demographic variables Also, longitudinal measurement in each single patient allows to assess fluctuation of biomarkers levels during the follow. Normative values for both NFL and GFAP will be used to interpret each result and perform group analyses. Moreover, T0 NFL and GFAP levels will be evaluated for their ability to predict functional test and PROs scoring worsening at sequent evaluation, as well as clinical/radiological worsening.

Objective 3. Early identification of the transitional phase from Relapse and Remitting (RRMS) to secondary progressive MS (SPMS), and subsequent patient monitoring.

This phase's main goal is to explore the ability of GFAP, together with NFL, of detecting the transitioning phase from RRMS to SPMS and the ability of monitoring the PIRA status.
We selected RRMS patients treated with Natalizumab (known to be highly effective on inflammation, but not on disease progression), and divided them into three groups: NEDA patients with No Evidence of Disease Activity, (NEDA), EDA (patients with Evidence of Disease (inflammatory) Activity (EDA) and PIRA) This phase of the project takes great advantage of the availability of more than 2000 serum samples of Natalizumab-treated RRMS patients stored in CRESM Biobank. Clinical records of possible candidates were studied to identify samples to be signed on one or the other group, with the objective of collecting around 50 samples per group.
Actually, 50 samples in the NEDA group, 17 samples in the EDA group and 14 patients in the PIRA group have been already selected and tested. For statistical analyses, GFAP and NFL levels will be evaluated for their ability, singularly or in combination, to correctly sort patients, when correlated with clinical and/or radiological findings. Also, they will be assessed as potentially predictive markers for PIRA.

e. Advancement in the field

- Indeed, the implementation of GFAP and NFL testing as a biological approach to add to the standard clinical assessments would guarantee accurate individual disease monitoring, therapy response evaluation and long-term prognosis prediction. Results will add knowledge to progressive MS patient management and establish biomarkers as disease measures in routine clinical practice.

- During the development of the project, quantitative measure of disease activity will possibly indirectly help revealing new pathogenesis mechanisms linking the biomarker levels to astrogliosis and neurodegenerative processes, to unveil biologically progressive patients in the very early phase of the disease and to correlate levels of subclinical inflammation (not related to MRI inflammatory activity or relapses) to subjective symptoms
- Surely, sensitive biomarkers able to early detect disease progression and to monitor treatment response are fundamental to personalize individual monitoring and treatment protocols. That said, adding GFAP and NFL levels to the patient monitoring panel can possibly help identifying sub-clinical non-responders and shift clinicians' attention to other interventions.

f. Publications

Bava CI, Valentino P, Malucchi S, Bottero R, Martire S, Sapio AD, Bertolotto A. Prevalence of elevated sNFL in a real-world setting: Results on 908 patients with different multiple sclerosis types and treatment conditions. Mult SclerRelatDisord. 2024 Aug;88:105748. doi: 10.1016/j.msard.2024.105748. Epub 2024 Jun 29. PMID: 38959590.

Malucchi S, Bava CI, Valentino P, Martire S, Lo Re M, Bertolotto A, Di Sapio A. In multiple sclerosis patients a single serum neurofilament light chain (sNFL) dosage is strongly associated with 12 months outcome: data from a real-life clinical setting. J Neurol. 2024 Dec;271(12):7494-7501. doi: 10.1007/s00415-024-12701-w. Epub 2024 Sep 23. PMID: 39313638.

5. Future directions and objectives for next years

a. Summary

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

b. Background and Significance

The cause of MS is unknown, but it has a presumed autoimmune etiology. Accordingly, pregnancy acts as modulator of disease activity. Unveiling the mechanism of the pregnancy-induced immunomodulation would lead to a better understanding of the MS pathogenesis and to the identification of novel potential therapeutic targets. Thanks to the collaboration with Prof. Luca Marozio, Head of the High Risk Pregnancy Unit and of the Research Laboratory of the Department of Surgical Sciences, Obstetrics and Gynaecology, University of Turin (Italy), and with Prof. Stefania Bruno of the Department of Medical Sciences and Molecular Biotechnology Center, University of Turin, we serum samples from healthy women. We tested 4 post-partum samples from these women at 24h, 48h, 72h and 10 days post-partum to measure NFL, GFAP, TAU and UCHL1. We are currently analizyng data to reveal the effects of birth type and parity (how many pregnancies before the one tested) on the biomarkers leves. This will help assess and understand mechanisms of physiological fluctuations of the biomarkers. Tests will be then carried out on MS women as well to assess differences and potential pathological mechanism of MS reactivation.
Moreover, we will carry on with GFAP determination to assess its role in clinical practice. In particular, we are proceeding to test samples from people with progressive MS forms at their diagnosis of clear progression, as well as every six months. Moreover, NFL and GFAP in combination are being tested in those patients with possible smoldering MS with not clear symptoms and signs upon the neurologists' request, to assess whether GFAP and NFL in combination can aid clinical decisions in terms of therapy escalation and progression definition.

Interestingly, NFL and GFAP are being tested on those patients with no specific therapy ongoing to monitor exacerbation of the disease. Lastly, NFL and GFAP are being assessed at initial diagnosis in all patients (ever relapse and remitting forms) to have baseline values and guarantee a personalized longitudinal monitoring in terms of biomarkers' increase compared to naïve state.

c. General aim and integration with mission of the Institute

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

I) deepening our knowledge on the pathophysiological processes occurring in a protective state of MS disease (pregnancy);

II) continuing on the path of improving the NFL and GFAP measure in the routine clinical monitoring of MS patients; implement individual personalized cut-off values for MS patients; obtain biomarkers profile in different clinical contexts, as during the switch to other therapies and during pregnancy; implement measurement service for the MS center network in the Northwest of Italy thanks to the service supported by Roche (see Grants);

III) setting up GFAP dosing; monitor disease progression of patients with primary progressive form of MS; identify and monitor relapsing-remitting MS patients transitioning to the secondary progressive form of MS;

IV) expanding CRESM biobank collection and distribution of biological samples/associated data for high quality research in the field of MS and other neurological disorders; creating a network of regional biobanks; implementing an appropriate process for the pediatric biobanking and new models for minor assent/consent; implementing an effective proper biobanking software for the management of samples and data: this is a crucial tool to enable the management of samples and data, to interface with the clinical management systems of the institution, according to privacy requirements and possibly to interface with external networks and platforms to facilitate the research and distribution of samples and data

e. Unique features of the project research

I) Our studies on serums from healthy pregnant women will help unravel mechanisms occurring right after delivery in a physiological way to determine if postpartum sNfL increase is associated to parturition per se. Moreover, to obtain more insights on nervous system involvement during the days around delivery, sNfL were quantified in parallel with 3 other nervous system biomarkers: Glial Fibrillary Acidic protein (GFAP), Tubulin Associated Unit (TAU) protein and Ubiquitin C-terminal Hydrolase L1 (UCHL1).
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**

"SR-X Ultra-Sensitive Biomarker Detection System" instrument (Quanterix) is a new instrument purchased by Clinical Neurobiology Laboratory and CRESM in 2018. The SR-X System is a benchtop instrument based on the innovative Simoa bead technology. This is based upon the isolation of individual immunocomplexes on paramagnetic beads using standard ELISA reagents. The main difference between

Simoa and conventional immunoassays lies in the ability to trap single molecules in femtoliter-sized wells, allowing for a “digital” readout of each individual bead to determine if it is bound to the target analyte or not.

The digital nature of the technique allows an average of 1000 times sensitivity increase over conventional assays with CVs less than 10 percent. This technology enables the ultra-sensitive detection of biomarkers in the range of subfemtomolar concentrations (below 1 pg/ml), in a variety of biological samples, including serum, plasma, cerebrospinal fluid (CSF), cell lysates.

The technology is currently being used for applications in a majority of therapeutic areas, including oncology, neurology, cardiology, inflammation and infectious disease. The SR-X is designed to support multiplexed detection of up to four biomarkers per sample, with low volume requirements to increase throughput and productivity while conserving precious samples.

In neurological field, this technology is widely used in different neurological disorders to measure NFL, proteins released following axonal damage in CSF, and also in blood, at very low concentrations. Thanks to its ultra-sensitivity, Simoa technology enables quantification of NFL also in blood, down to concentrations occurring in healthy persons. Several other neurological biomarkers can be assessed by Simoa technology on SR-X instrument including GFAP, TAU, Ab42, Ab40, alpha-sinuclein. In addition, the technology enables to set-up custom assays, when specific antibodies are available for the analyte of interest.

NeuroWebinar & Seminar 2024

1 appointment per week, on Friday at 2.00 pm

**Hybrid seminar*: both in presence and on webex*

Friday 12/1/2024 h. 2.00 pm - Hybrid Seminar

Filippo Sean Giorgi, Dept. Translational Research and of New Surgical and Medical Technologies, University of Pisa

The central noradrenergic system and neurodegeneration occurring along the Alzheimer's Disease continuum and ageing

Understanding Alzheimer's Disease (AD) pathophysiology represents a major challenge of neuroscience research, and effective disease-modifying therapies are still far to be developed. In recent years, growing attention has been focused on the possible role of the noradrenergic nucleus Locus Coeruleus (LC) in AD pathogenesis and physiopathology. Experimental findings and human post-mortem data all converge in underscoring the impact of early LC degeneration in AD-related degenerative phenomena and AD natural history. A better shaping of the features and role of LC in AD might be crucial in detailing its role as a research biomarker, as well as a potential therapeutic target of AD.

In this seminar, an overview of the current research on the role of the LC in AD will be provided, including some pieces of data I have been collecting in recent years. In particular, we, in parallel with other groups, have been able to confirm in vivo in humans the significant involvement of LC in the AD continuum by profiting of advanced Magnetic Resonance Imaging (MRI) tools (LC-MRI) which have been developed ad-hoc. Recently, we have also shown its involvement in the conversion from Mild Cognitive Impairment to dementia and the association between the degeneration of different parts of LC with the cortical metabolism of AD patients. Moreover, we explored the relationship between LC structural integrity and neuroinflammation, by assessing the association of LC-MRI with plasma interleukins in patients and healthy controls. Finally, I will illustrate and discuss experimental studies currently ongoing on the relationship between LC and normal ageing, and between LC degeneration and late-onset epilepsy; these might offer additional perspectives for dissecting the complex pathophysiology of AD and the role of LC.

Host: Alessandro Vercelli

Friday 19/1/2024 h. 9.00 am - Webinar

Makoto Sato, Department of Anatomy and Neuroscience, Graduate School of Medicine; Division of Child Development, United Graduate School of Child Development (UGSCD) - Osaka University, JAPAN

Cytoskeletons and cortical development: How does the neocortex develop to establish the prototype of neuronal circuits by neuronal migration and collateral formation?

To understand the complex neuronal circuits for higher functioning of the neocortex from a compositional perspective, I have studied cortical development, in particular cytoskeletal regulatory mechanisms underlying migration and collateral formation. Periventricular nodular heterotopia gave me the first hint to study cortical development focusing on the regulation of cytoskeletons. Periventricular heterotopia is a hereditary disease in which the brain has a second cortex (cluster of nerve cells) around the ventricle, a so-called double cortex, and one of its characteristics is intractable epilepsy.The cause of the disease is believed to be a mutation in the actin-binding protein filamin A on the X chromosome, suggesting that filamin A is important for neurons to migrate out of the cortical ventricular zone to form the neocortex. We have identified and studied a novel molecule, FILIP (filamin A interacting protein), which promotes the degradation of filamin A.

Very recently, it was reported that mutations in FILIP (FILIP1 in human) cause congenital arthrogryposis multiplex, intellectual disability, holoprosencephaly, and encephalocele in human (FILIP disease). In my talk, I will introduce a series of FILIP-related studies to and its regulatory factors, including some unpublished data.
It is generally believed that mutation of molecules involved in neuronal migration increases susceptibility to various neuropsychiatric diseases, but the relationship between these mutations has not been fully elucidated. Therefore, to examine changes in neural networks due to variations in neuronal arrangement, we first constructed a system to visualize single-cell level neural networks for individual cerebral cortical neurons. Sequential collateral formation to apparently predetermined targets is critical to establish the prototype of neuronal circuits. I will also present our latest results that underlie such collateral formation in my talk.

Host: Alessandro Vercelli

Monday 22/1/2024 h. 2.00 pm - Hybrid Seminar

Elia Di Schiavi, Institute of Biosciences and BioResources, IBBR; Dept. Biology, Agriculture and Food Science, CNR Naples, Italy

Splicing regulation of Reticulon is involved in preventing neurodegeneration in a C. elegans model of SMA

An efficient splicing of mRNA is required in all cells, but neurons seem to be more vulnerable to splicing perturbations. In fact, numerous neurodegenerative diseases are caused by splicing defects, including Spinal Muscular Atrophy (SMA). However, why neurons are more affected to splicing alterations and which step of the RNA processing is impaired in this disease is still debated. SMA is caused by mutations in the Survival Motor Neuron (Smn) gene, which is involved in RNA metabolism and splicing. We have demonstrated that genes differentially expressed or spliced in induced pluripotent cell-derived motor neurons (iPS-MNs) from SMA patients are enriched in the RNA motif 7. This motif is specifically recognized by hnRNPQ, a spliceosomal component physically interacting with SMN. We demonstrated that hrpr-1, the hnRNPQ homolog in C. elegans, is involved in motoneurons (MNs) survival similarly to smn-1, the Smn homolog. We demonstrated that they genetically interact and exert a neuroprotective function specifically in MNs. Comparing hrpr-1 known targets in C. elegans and the alternatively spliced genes identified in SMA patients, we identified a new possible downstream target of the pathway: ret-1, the only homolog in C. elegans of Reticulon genes, a family of transmembrane proteins involved in vesicle recycling and formation, and in neurite outgrowth. We confirmed a possible involvement of ret-1 in SMA by observing alteration in its transcript levels in C. elegans, SMA mice and patients. Moreover, we demonstrated that ret-1 splicing pattern is altered when smn-1 is depleted and that hrpr-1 and smn-1 work together to guarantee the correct splicing of exon 5 of ret-1 gene. Thus, we identified for the first time a neuroprotective role of hrpr-1 and the involvement of ret-1 in neurodegeneration.

Piera Smeriglio, Center of Research in Myology, Sorbonne University, Paris, France

Deciphering key molecular players in skeletal muscle affected by SMA

Spinal Muscular Atrophy (SMA) is traditionally considered a disease of the motor neurons, however, increasingly the systemic role of the SMN protein is being underscored. In particular, the role of the muscle as both an axis of pathology and driver of overall disease, is being appreciated. After an initial characterization of the phenotypic and molecular features of the skeletal muscle tissue in a severe SMA mouse model, we sought to investigate the response of the muscle upon administration of the approved therapies. Therefore, we collected paravertebral muscle from SMA Type II patients (n=8) after treatment with Nusinersen and age matched controls (n=7) and performed RNA-sequencing. This analysis revealed a heterogeneous response of the skeletal muscle tissue to the therapy with most of the patients having a persistent DNA damage and P53 pathways activation despite the restoration of SMN levels. This study provides a molecular roadmap of the state of SMA muscle after treatment. Work is ongoing to determine that

molecular reasons – be they genetic, epigenetic, or clinical for the heterogeneous response to Nusinersen injection, and to test drug candidates to improve mitochondrial function and decrease DNA damage in skeletal muscle.

Host: Marina Boido

Friday 2/2/2024 h. 2.00 pm - Webinar

Ariel Di Nardo, CNRS Research Scientist and Co-director, Development & Neuropharmacology Team, CIRB, Collège de France

Anxiety-like behavior regulated by non-cell autonomous transcription factor activity

Our laboratory investigates the role of non-cell autonomous homeoprotein transcription factors in regulating cerebral cortex physiology. We discovered that OTX2 homeoprotein is expressed in the choroid plexus, secreted into cerebrospinal fluid, and transferred into parvalbumin (PV)-expressing interneurons in mice. OTX2 participates in PV cell maturation and regulates the timing of plasticity critical periods throughout the brain. These juvenile periods allow for remodeling of circuitry in response to the environmental and genetic contexts, and are associated with disease outcomes. Although our initial OTX2 studies were primarily focused on mouse visual system critical periods, we have also investigated higher order circuits involved in anxiety-like behavior shaped by early-life stress. Our recent findings revealed OTX2 target genes in cortical PV cells with epigenetic outcomes and showed that choroid plexus OTX2 affects animal behavior.

Host: Serena Stanga

Friday 9/2/2024 h. 2.00 pm - Hybrid Seminar

Alessandro Usiello, Dept. Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania L. Vanvitelli

D-amino acids metabolism abnormalities in neurological and psychiatric disorders

D-aspartate (D-Asp) has a transient emergence in the mammalian brain. It is abundant in the embryonic phase and the first post-natal days before significantly decreasing thereafter. Interestingly, during prenatal phases, the intracellular localization of D-Asp seems to be developmentally regulated, according to the functional activity of neuroblasts. It has long been established that D-aspartate oxidase (DDO) is the enzyme responsible for D-Asp catabolism. Accordingly, the post-natal decrease of D-Asp content is associated with the concomitant, progressive increase in *Ddo* gene expression and DDO activity in the rodent brain. D-Asp is present at extracellular level, where it acts as an agonist at NMDA and mGlu5 receptors. In line with its pharmacological role, we found that adult mice with abnormally high cerebral D-Asp levels showed increased NMDA receptor-dependent functional and structural plasticity, and improved spatial memory.
Although these findings highlight the influence of non-physiologically high D-Asp levels on several cerebral processes at adulthood, it is so far unknown the significance of embryonic D-Asp in the mammalian brain and its involvement on brain functions and behaviors at adulthood. To clarify this issue, we have recently generated a novel knockin mouse model in which the expression of DDO is anticipated starting from the zygotic stage to enable the removal of the embryonic storage of cerebral D-Asp. To this aim, we targeted a *Ddo* cDNA cassette in the genomic *Rosa26* locus to allow the ectopic transcription of *Ddo* under the regulatory control of the constitutive *Rosa26* promoter. We found that knockin strategy resulted in a strong, allele-dependent increase of both *Ddo* expression and DDO enzymatic activity in heterozygous ($R26^{Ddo/+}$) and homozygous ($R26^{Ddo/Ddo}$) *Ddo* knockin brains, compared to wild-type controls ($R26^{+/+}$). These molecular alterations resulted in a corresponding strong ontogenetic depletion of cerebral D-Asp, from embryonic to adult phase. However, deregulated *Ddo* gene expression did not affect the cerebral levels of L-Asp, the precursor of D-Asp biosynthesis, as well as the metabolism of D-serine and L-glutamate, the two main neuroactive molecules involved in NMDA receptor-dependent transmission. Surprisingly, despite the removal of embryonic cerebral D-Asp, *Ddo* knockin mice were viable, fertile and did not show any evident abnormalities at adulthood. Moreover, histological and immunohistochemical analysis revealed no gross differences in brain size or structural organization and no variations in neuronal density and distribution in adult *Ddo* knockin mice. Conversely, we found that early D-Asp depletion was associated with increased number of cortical parvalbumin-positive interneurons and improved cognitive abilities of adult *Ddo* knockin mice in spatial memory e recognition tasks. Overall, the molecular, morphological and behavioral characterization of *Ddo* knockin mice revealed unexpected phenotypes that deserve further investigations not only in adult but also in juvenile and embryonic phases of mouse brain development.

Host: Alessandro Vercelli

Friday 16/2/2024 h. 2.00 pm - Hybrid Seminar

Vasco Meneghini, San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), IRCCS San Raffaele Scientific Institute, Milan, Italy

Targeting astrocytes with editing technologies to treat Alexander Disease

Alexander disease (AxD) is a rare, lethal leukodystrophy caused by gain-of-function mutations in the gene encoding for glial fibrillary acidic protein (GFAP), the main intermediate filament of astrocytes. Accumulation of GFAP aggregates in Rosenthal fibers leads to central nervous system (CNS)dysfunction with typical pathological traits such as astrogliosis, loss of myelin, seizures, and spasticity. No cure is currently available for this neurodegenerative disorder.

We developed a novel, single-dose gene editing strategy for the lifetime treatment of AxD. We selected a single guide RNA (gRNA) targeting the murineGfapgenein3T3 cells transduced with a lentiviral vector (LV) harboring the R76H-mutant GFAP protein fused to mCherry. FACS analysis of mCherry expression showed that the best gRNA candidate induced a robust knock-down ofGFAP-mCherry, while nogene editing at top off-target loci was evident. To optimize the in vivo brain-directed delivery of theGfap-targeting CRISPR system, pilot experiments defined the optimal injection protocol, AAV serotype and promoter, resulting in high astrocytic tropism and transduction rates of AxD-affected brain regions. Selected AAV carrying the Gfap-targetinggRNA and the Cas9 nuclease was administered by intracerebroventricular injections in neonatal AxD mice. AVV-mediated Cas9/sgRNAdelivery resulted in on-target editingin GFAP+ astrocytes, decreased astrogliosis and reduced accumulation of Rosenthal fibers - a hallmark of AxD pathology - in white matter regions. These data provide in vivo proof-of-concept of the efficacy of a CRISPR/Cas9 editing approach in ameliorating disease-associated phenotypes.

To expand on the potential of gene editing as a mutation-specific treatment for AxD, we are currently developing allele-specific gene therapies targeting the murine R76H mutation, homolog of the human mutation hotspot detected in AxD patients. Among them, we identified adenine base editors that efficiently correct the Gfap mutation in vitro and we are currently validating this approach in vivo. Overall, our study provides initial proof-of-concept data on the efficacy of a CRISPR/Cas9 editing approach in ameliorating disease-associated phenotypes. Our results pave the way for pre-clinical studies aimed at improving the editing tolls targeting the mutated Gfap allele in the CNS using AAV vectors or, prospectively, non-viral delivery systems.

Host: Martina Lorenzati

Friday 23/2/2024 h. 2.00 pm - Hybrid Seminar

Helena L. A. Vieira, UCIBIO, Applied Molecular Biosciences Unit, Department of Chemistry, NOVA School of Science and Technology, Universidade Nova de Lisboa, Portugal

Carbon monoxide promotes mitochondrial homeostasis in brain cells: Cell energy and fate control in stroke context

Carbon monoxide (CO) is a gasotransmitter endogenously produced by the activity of heme oxygenase, which is a stress-response enzyme. Endogenous CO or low concentrations of exogenous CO have been described to present several cytoprotective functions: anti-apoptosis, anti-inflammatory, vasomodulation, maintenance of homeostasis, stimulation of preconditioning and modulation of cell differentiation.

The seminar will present and discuss how CO is cytoprotective in glial cells and how CO improves neuronal differentiation. In fact, COprevents oxidative stress-induced astrocytic cell death by improving oxidative metabolism [1] and mitochondrial quality control [2]. The anti-neuroinflammatory effect of CO is also dependent on microglial metabolism control regulated by neuroglobin [3]. Finally, neuronal differentiation is facilitated by CO modulation of metabolism: oxidative phosphorylation [4] and pentose phosphate pathway [5].

[1] A.S. Almeida, C.S.F. Queiroga, M.F.Q. Sousa, P.M. Alves, H.L.A. Vieira, Carbon monoxide modulates apoptosis by reinforcing oxidative metabolism in astrocytes: Role of Bcl-2, J. Biol. Chem. 287 (2012) 10761–10770. doi:10.1074/jbc.M111.306738.

[2] C. Figueiredo-Pereira, B. Villarejo-Zori, P.C. Cipriano, D. Tavares, I. Ramírez-Pardo, P. Boya, H.L.A. Vieira, Carbon Monoxide Stimulates Both Mitophagy And Mitochondrial Biogenesis to Mediate Protection Against Oxidative Stress in Astrocytes, Mol. Neurobiol. 60 (2023) 851–863. doi:10.1007/s12035-022-03108-7.

[3] D. Dias-Pedroso, J.S. Ramalho, V.A. Sardão, J.G. Jones, C.C. Romão, P.J. Oliveira, H.L.A. Vieira, Carbon Monoxide-Neuroglobin Axis Targeting Metabolism Against Inflammation in BV-2 Microglial Cells, Mol. Neurobiol. 59 (2022) 916–931. doi:10.1007/s12035-021-02630-4.

[4] A.S. Almeida, U. Sonnewald, P.M. Alves, H.L.A. Vieira, Carbon monoxide improves neuronal differentiation and yield by increasing the functioning and number of mitochondria, J. Neurochem. 138 (2016)

423–435. doi:10.1111/jnc.13653.
[5] A.S. Almeida, N.L. Soares, C.O. Sequeira, S.A. Pereira, U. Sonnewald, H.L.A. Vieira, Improvement of neuronal differentiation by carbon monoxide: Role of pentose phosphate pathway, Redox Biol. 17 (2018) 338–347. doi:10.1016/j.redox.2018.05.004.

Host: Alessandro Vercelli

Friday 1/3/2024 h. 2.00 pm - Hybrid Seminar

Marco Terenzio, OIST - Okinawa Institute of Science and Technology, Japan

Regulation of RNP granule dynamics and axonal translation in sensory and motor neurons

Neurons are highly polarized cells with an elongated axon that extends far away from the cell body. In order to maintain neuronal homeostasis, neurons rely extensively on axonal transport of membranous organelles and other molecular complexes in addition to local translation of proteins. Axonal transport plays a central role in the establishment of neuronal polarity, axonal growth and stabilization and synapses formation, allowing for precise spatio-temporal activation and modulation of numerous molecular cascades. Anterograde and retrograde axonal transport are supported by various molecular motors, such as kinesins and dyneins, and a complex microtubule network. In this seminar I will discuss some aspects of retrograde signaling in neurons, ranging from injury signals to dynein-mediated axonal transport, which are critical for the survival of neurons. We will also discuss the storage and translation of mRNA granules in axons and strategies to promote axonal regeneration through the use of specialized substrates and the tools we have developed to investigate the mechanisms underlying axonal degeneration in Amyothrophic Lateral Sclerosis (ALS).

Host: Letizia Marvaldi

Friday 15/3/2024 h. 2.00 pm - Webinar

Ferdinand Althammer, Heidelberg University Hospital, Institute for Human Genetics, Germany

Microglial Angiotensin II signaling in cardiovascular diseases

Heart failure (HF) is a widespread and debilitating condition impacting over 64 million individuals globally. Beyond compromised cardiovascular function and related systemic issues, HF patients commonly experience depression and significant cognitive decline. Despite the presence of neuroinflammation and brain hypoperfusion in both humans and rodents with HF, the specific neuronal substrates and mechanisms contributing to cognitive deficits remain elusive.

To address this knowledge gap, we employed a well-established HF rat model replicating clinical outcomes and employed a multidisciplinary approach spanning behavioral, electrophysiological, neuroanatomical, molecular, and systemic physiological analyses. Our investigations revealed neuroinflammation, hypoperfusion/hypoxia, and neuronal deficits in the hippocampus of HF rats, correlating with disease progression. Increased expression of Ang II receptor type 1a (AT1aRs) in hippocampal microglia preceded neuroinflammation onset. Blocking AT1Rs with the therapeutic drug Losartan efficiently reversed neuroinflammatory endpoints, improving cognitive performance in HF rats. Additionally, we demonstrated that circulating Ang II could access the hippocampal parenchyma in HF rats, potentially initiating the neuroinflammatory cascade.

This study identified the hippocampus as a crucial neuronal substrate, Ang II–driven neuroinflammation as a key mechanism, and AT1aRs as a potential neuroprotective therapeutic target for treating cognitive deficits in HF. The findings underscore the significance of understanding the interplay between microglia and local microvasculature, revealing an impact on blood-brain barrier integrity and cerebral blood flow regulation during HF. In our ischemic HF rat model, increased vessel-associated microglia (VAM) in HF rat hippocampi exhibited heightened Ang II AT1a receptor expression.

Acute Ang II administration induced microglia recruitment to the perivascular space, emphasizing the role of microglia-vascular interactions in HF-induced neuroinflammation. Administering an AT1aR blocker to HF rats prevented microglia recruitment to the perivascular space, normalizing levels to those in healthy rats. These results unveil novel therapeutic avenues targeting microglia-vascular interactions to mitigate neuroinflammation in cardiovascular diseases, providing valuable insights into the pathophysiology of this prevalent condition.

Host: Ilaria Bettocchi

Friday 22/3/2024 h. 2.00 pm - Hybrid seminar

Silvia Diviccaro, Dipartimento di Scienze Farmacologiche e Biomolecolari - Università di Milano

GUT-MICROBIOTA-BRAIN AXIS: FOCUS ON GUT STEROIDS

Sex steroids, derived mainly from gonads, can shape gut microbiota composition. Therefore, it is not surprising that sexual dimorphic features dictated by sex steroids also concern microbes [1-3]. The gut microbiome as well as its metabolites actively participate in host homeostasis prominently in intestinal health and brain response via the gut-microbiota-brain axis (GMBA), a bidirectional communication that includes immune, endocrine, neural, and humoral routes. Thus, in GMBA, the involvement of steroid molecules is plausible and should be highlighted. Importantly, the total lack of microbiota in axenic experimental models drastically influences steroid levels both in plasma and in the brain, regardless of where the molecules are synthesized.

However, to take into consideration peripheral steroidogenic glands and the brain as exclusive steroidogenic centers is limited. The gastrointestinal tract has a strong ability to synthesize glucocorticoids in inflammatory conditions and, as demonstrated more recently, the synthesis of other steroids such as the first precursor (i.e., pregnenolone), estrogens (i.e., 17beta-estradiol), testosterone, progesterone, and their active metabolites, such as dihydrotestosterone and allopregnanolone. The pattern of intestinal steroid levels (i.e., gut steroids) is sexually dimorphic and is maintained after gonadectomy, suggesting a significant gut steroid pool locally acting by steroid receptors, such as GABA-A receptor.

Interestingly, intestinal steroidogenesis and gut steroid levels do not reflect the brain environment in some pathological conditions, suggesting that the gut and brain may be differently affected. In particular, in type 1 diabetes mellitus as well as after treatment with a steroidogenic inhibitor (i.e., finasteride), or a selective-serotonin reuptake inhibitor (i.e., paroxetine) gut steroid levels are affected in animal models. Of note, the steroid alterations were coupled with gut microbiota alterations, which were also observed in patients affected by these disorders, highlighting a putative dysfunction of GMBA. Bearing in mind that the gut and brain constantly send messages to each other and are influenced by microbiota will be also crucial to investigate how steroids influence these three different compartments in physiopathological conditions.

Host: Marilena Marraudino

Friday 5/4/2024 h. 2.00 pm - Hybrid seminar

Deborah Chiabrando, Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center “Guido Tarone”, University of Torino

Dysregulation of FLVCR1-dependent mitochondrial calcium handling in neural stem cells causes congenital hydrocephalus.

Congenital hydrocephalus (CH), occurring in approximately 1/1000 live births, represents an important clinical challenge due to the limited knowledge of underlying molecular mechanisms. The discovery of novel CH-genes is thus essential to shed light on the intricate processes responsible for ventricular dilatation in CH. Here, we identify FLVCR1 (Feline Leukemia Virus Subgroup C Receptor 1) as a novel gene responsible for a severe form of CH in humans and mice.
Mechanistically, our data reveal that FLVCR1a interacts with the IP3R3-VDAC complex located on mitochondria-associated membranes (MAMs) that controls mitochondrial calcium handling. Loss of Flvcr1a in mouse neural stem cells (NSCs) affects mitochondrial calcium levels and energy metabolism, leading to defective cortical neurogenesis and brain ventricle enlargement. These data point to defective NSC calcium handling and metabolic activity as one of the pathogenetic mechanisms driving CH.

Host: Enrica Boda

Friday 19/4/2024 h. 2.00 pm - Hybrid seminar

Pierre J. Magistretti, MD, PhD - Vice President for Research
Division of Biological and Environmental Sciences and Engineering, KAUST, Thuwal, Saudi Arabia

Neuron-glia metabolic coupling: role in neuronal plasticity and neuropsychiatric disorders

A tight metabolic coupling between astrocytes and neurons is a key feature of brain Energy metabolism (Magistretti and Allaman, Neuron, 2015). Over the years we have described two basic mechanisms of

neurometabolic coupling. First the glycogenolytic effect of VIP and of noradrenaline indicating a regulation of brain homeostasis by neurotransmitters acting on astrocytes, as glycogen is exclusively localized in these cells. Second, the glutamate-stimulated aerobic glycolysis in astrocytes. Both the VIP-and noradrenaline-induced glycogenolysis and the glutamate-stimulated aerobic glycolysis result in the release of lactate from astrocytes as an energy substrate for neurons (Magistretti and Allaman, Neuron, 2015; Magistretti and Allaman, Nat Neurosci Rev, 2018).
We have subsequently shown that lactate is necessary not only as an energy substrate but also as a signaling molecule for long-term memory consolidation, for maintenance of LTP and for dendritic spine dynamics (Suzuki et al, Cell, 2011; Vezzoli et al, Cerebral Cortex, 2019). At the molecular level we have found that L-lactate stimulates the expression of synaptic plasticity-related genes such as Arc, Zif268 and BDNF through a mechanism involving NMDA receptor activity and its downstream signaling cascade Erk1/2 (Yang et al, PNAS, 2014). A transcriptome analysis in cortical neurons has shown that the expression of a total of 20 genes is modulated by L-Lactate; of these, 16 involved in plasticity and neuroprotection are upregulated and 4 involved in cell death are down regulated (Margineanu et al. Front in Mol Neurosci, 2018). This set of results reveal a novel action of L-lactate as a signaling molecule in addition to its role as an energy substrate (Magistretti and Allaman, Nat Neurosci Rev, 2018).

These actions of L-Lactate are also relevant for animal models of neuropsychiatric disorders. Indeed we have shown that peripheral administration of lactate exerts antidepressant-like effects in three animal models of depression (Carrard et al, Mol.Psy., 2016 and 2021). Finally, we have shown that the transfer of L-Lactate from astrocytes to neurons plays a key role in an appetitive memory task involving the basolateral amygdala such as cocaine place preference in mice (Boury-Jamot et al. Mol Psy, 2016).Recently, using electrophysiology, two-photon imaging, cognitive tasks, and mathematical modeling, we have shown that both glucose and lactate are involved in engram formation, with lactate supporting long-term synaptic plasticity evoked by high-stimulation load activity patterns and high attentional load in cognitive tasks while glucose is sufficient for less demanding neural computation and learning tasks (Dembritskaya et al, PNAS, 2022).
In view of the critical role of astrocytes in the regulation of brain energy metabolism that we have explored over the past four decades, and of the evidence that dysregulation of astrocyte-mediated metabolic pathways is involved in brain hypometabolism, we are now focusing on pharmacologically targeting astrocytes to address the therapeutic needs in neuropsychiatric disorders characterized by hypometabolism. We have gathered recent evidence in preclinical animal models of genetic diseases such as for example Glucose Transporter 1 Deficiency Syndrome (GluT1DS or De Vivo disease) and neurodegenerative diseases such as Alzheimer's disease that targeting astrocytes to overcome brain hypometabolism opens promising therapeutic avenues (Beard et al 2022, Front in Physiol).

Host: Corrado Calì

Friday 3/5/2024 h. 2.00 pm - Hybrid seminar

Dustin J. Penn and Sarah M. Zala - Konrad Lorenz Institute of Ethology, University of Veterinary Medicine, Vienna - Austria

Courtship vocalisations of wild house mice are highly dynamic and influence copulatory success

We analysed the courtship vocalisations of wild house mice (F1wild-caught *Mus musculus musculus)* emitted during different stages of courtship and mating, and we tested whether their calls predict male copulatory success. We recorded their behaviour and vocalisations over 40 h and analysed 40 - 50 min of recordings per pair. Of the ca. 53 000 vocalisations, 87% were ultrasonic (USVs), which we classified into 11 different syllable types, and 10% were partly audible broadband (BBVs) vocalisations; often called 'squeaks'. We found that the mice emitted a distinctive vocal repertoire and composition during each phase of courtship, and that their calls became increasingly complex over stages of courtship, especially once males began female mounting. During copulatory behaviour, USVs and BBVs (probably emitted by males and females respectively) became closely timed and uttered in tight synchrony, much like duetting birds. Approximately 40% of males copulated with ejaculation during the study and we found several differences between the vocalisations of the pairs that successfully copulated versus non-copulating pairs. USV emission increased during male sexual behaviours, and especially among the mice that successfully copulated. Our results show that the courtship vocalisations of wild house mice are much more complex and dynamic than has been assumed and they provide the first evidence for vocalisations that influence copulatory success.

Host: Serena Bovetti

Friday 10/5/2024 h. 2.00 pm - Webinar

Jeroen Pasterkamp, Utrecht University Medical Centre and Utrecht University

Modelling neurodegenerative disorders in a dish

The goal of our work is directed towards understanding how neural circuits form during development and why they change or degenerate during disease. For this research, we use a combination of molecular cell biological approaches (e.g. scRNAseq, CRISPR), (3D) microscopy, mouse genetics and iPSC-based modelling in combination with microfluidics. Here I will focus on our work that attempts to dissect the molecular mechanisms underlying neurodegenerative diseases, in particular amyotrophic lateral sclerosis (ALS). ALS is a fatal neurodegenerative disorder with a lifetime risk of 1:400, affecting upper and lower motor neurons. Loss of motor nerves leads to weakness of skeletal muscles, ultimately resulting in death 3-5 years after diagnosis. Treatment options for ALS are limited and the development of new therapeutic strategies requires further insight into the pathogenic mechanisms underlying ALS.
In addition to employing ALS animal models, we have invested in setting up a wide array of advanced *in vitro* systems generated from human induced pluripotent stem cells (iPSCs) in combination with sensitive readouts. These models range from individual cell types, such as motor neurons or skeletal muscle cells, to combinations of cell types in microfluidic devices and even engineered 3D tissues (organoids). We have developed several neural organoid protocols for analyzing 3D neural tissue and specific cell-cell interactions. Importantly, these models show established pathological hallmarks of ALS as well as pathogenic changes and can therefore be used to further dissect disease mechanisms and to identify therapeutic targets.

Host: Roberta Schellino

Friday 31/5/2024 h. 2.00 pm - Hybrid seminar

Michèle Studer, Institute of Biology Valrose, iBV; Univ. Côte d'Azur (UCA)

In vitro and in vivo modelling of an emerging neurodevelopmental disorder

My team is interested in understanding the relationships between impaired cortical development, malformations, and consequent symptoms in neurodevelopmental disorders, as well as the genes implicated in these processes. BBSOAS (Boonstra-Bosch-Schaff Optic Atrophy Syndrome) is a recently described monogenic neurodevelopmental disorder caused by the haploinsufficiency of the NR2F1 gene, a transcriptional regulator playing a key role during brain development. Intellectual disability, autistic traits, and visual impairments are the most common symptoms affecting BBSOAS patients although with heterogenous levels of severity.
By employing a multidisciplinary approach including disease animal models, 3D organoids, genetic manipulation, -omics approaches as well as structural bioinformatics, we are starting to understand the impact of the different mutations on protein stability and cell function and contribute to unraveling the genotype/phenotype correlation of the disease.

Host: Silvia De Marchis

Wednesday 5/6/2024 h. 12.00 am - Hybrid seminar

Elisa Galliano, University of Cambridge, UK

Neuronal heterogeneity and plasticity in the olfactory bulb

Dopaminergic neurons in the olfactory bulb regulate early sensory processing by adjusting synaptic gain and exhibiting remarkable plasticity. Recent findings highlight their diverse responses to sensory deprivation, with some altering structure and excitability while others rely on synaptic changes. This plasticity is more pronounced than that displayed by excitatory neurons, suggesting rapid adaptation for sensory processing. Our lab's current work aims to understand how these responses contribute to generating appropriate neuronal outputs at both network and behavioural levels.

Host: Silvia De Marchis

Friday 5/7/2024 h. 2.00 pm - Hybrid seminar

Angelisa Frasca, Dipartimento di Biotecnologie Mediche e Medicina Traslazionale - Università di Milano

Neural precursor cells rescue symptoms of Rett syndrome by activation of the Interferon γ pathway

MECP2 mutations cause Rett syndrome (RTT), the first cause of severe intellectual disability in girls. Neural Precursor Cell (NPC) transplantation was proved safe and efficacious in many neurological disorders, including autism. Willing to respond to the unmet need of a cure for RTT, during these years our research group investigated the therapeutic potential of adult NPCs in *Mecp2*deficient mice, modelling RTT. Although the prime mechanism of action of NPCs is the replacement of damaged cells, transplanted cells also exert their benefits through a bystander mechanism. Indeed, by sensing the pathological environment, they promote immunomodulation, neuroprotection and brain plasticity through the secretion of several molecules. Moreover, transplanted NPCs adapt their fate and functions to the specific pathological context and can engage in a rich talk with resident cells. I will present you our data demonstrating that by sensing the pathological context, NPC-secreted factors induce the recovery of morphological and synaptic defects typical of *Mecp2* deficient neurons and that NPC transplantation prolongs the lifespan of *Mecp*2 null mice, restoring memory and motor functions. To gain insight into the involved molecular mechanism, byRNA-seq study we have disclosed the involvement of the Interferon (IFN)γ pathway. Coherently, the IFNγrecombinant molecule was effective in reverting motor and cognitive impairmentsin *Mecp2* null animals and in improving synaptic alterations of RTT neurons. Together, our data provide the "proof of concept" of a NPC-based therapy for RTT and indicate the involvement of IFNγ,thereby suggesting this molecular pathway as a potential therapeutic target for RTT.

Host: Enrica Boda

Tuesday 17/9/2024 | h. 5.00 - 6.00 pm

Joint Seminar NICO & Department of Neuroscience, University of Turin

MBC - Via Nizza 52, Torino | Aula Darwin

Manuel Valiente, Group Leader, Brain Metastasis Group - Department of Molecular Oncology Program, Spanish National Cancer Research Centre (CNIO)

A novel strategy to challenge resistance of symptomatic brain metastasis to immune checkpoint blockade

Host: **Prof. Roberta Rudà - Prof. Alessandro Vercelli**

Friday 27/9/2024 h. 2.00 pm - Webinar

Tudor Constantin Badea, Scientific Director, Research and Development Institute, Faculty of Medicine, Transilvania University of Brasov, Romania & Research Scientist (CS II) National Brain Research Centre, Research Institute for Artificial Intelligence, Romanian Academy, Bucharest, Romania

Genetic analysis of neuronal circuits with cell type resolution: development, function and pathology.

Knowledge of cell type composition is crucial for the understanding of the development , function, pathology and repair of the nervous system. The definition of a cell type can comprise anatomic/morphologic, electrophysiologic and molecular features, as well as knowledge about its function in the functioning of the entire circuit. While deep sequencing has greatly accelerated our capability of classifying neuronal cell types using their gene expression profiles, the complete definition of the cell type requires understanding of all features of the neuron. We approach this problem by generating conditional knock-in reporter alleles in mice and using combinatorial genetic approaches in order to target individual cell types and characterize their combined features. Specifically we are interested in Retinal Ganglion Cells, the neurons that carry visual information from the eye to the brain. I will discuss our efforts for RGC classification and molecular characterization including interesting new RGC markers with implications for metabolic neuropathies.

Host: Silvia De Marchis

Friday 25/10/2024 h. 2.00 pm - Hybrid seminar
SEMINAR CYCLE of the PhD in Neuroscience of Turin

Fabio Benfenati, University of Genoa, Director of Research at the Italian Institute of Technology (IIT)

A membrane-targeted photoswitch restores physiological retinal processing in the degenerate

The lack of effective therapies for visual restoration in Retinitis pigmentosa and macular degeneration has pushed the scientific community to pioneer therapeutical strategies to replace dead photoreceptors, including optogenetics and retinal prostheses. However, the resulting visual restoration is poor. Here, we show that a recently characterized membrane-targeted photoswitch, Ziapin2, is capable of reinstating, in degenerate retinas, the complexity of the physiological responses to light stimuli that are implemented by a healthy retina. We tested the ex vivo effects of Ziapin2 on blind retinal explants from rd10 mice and RCS rats, two distinct genetic models of photoreceptor degeneration, by recording light-evoked responses from retinal ganglion cells (RGCs) with patch clamp and high- density multielectrode arrays. Thanks to its dual effect on intrinsic excitability, Ziapin2 reinstated brisk and sluggish ON, OFF, and ON-OFF responses in RGCs evoked by full-field or pattered stimuli, accompanied by the reactivation of excitatory and inhibitory conductances impinging on RGCs. When tested in vivo, a single intravitreal injection of Ziapin2 in fully blind 6-month-old rd10 mice restored light-driven behavior and optomotor reflexes, with a concomitant activation of RGC populations similar to sighted animals. The results indicate that Ziapin2 is a promising molecule for reinstating physiological visual responses at late stages of retinal degeneration, irrespective of the mutation causing degenerative blindness.

Host: Serena Bovetti

Friday 15/11/2024 h. 2.00 pm - Hybrid seminar
DATA REPORT

Giulia Nato & Marco Fogli, Research Group NICO Adult Neurogenesis

The widespread neurogenic potential of the adult brain

The mature mammalian brain lacks regenerative capacity, and it has long been thought to lack a stem cell (SC) compartment.Nonetheless, it is now known that neurogenesis extends to postnatal development with some variations across species and brain regions, and in two specialized niches, the ventricular-subventricular zone (V-SVZ) and the subgranular zone (SGZ) it persists also during adulthood. In these regions subpopulation of astrocytes act as neural stem cells (NSCs) actively producing neurons through life. This organization of the adult brain NSC potential is aligned with established models in SC research whereby SCs are rare and anatomically restricted cells. Furthermore, the SVZ and DG progenitors are committed to the production of olfactory bulb (OB) interneurons and dentate gyrus (DG) granule cells, indicating a very limited neurogenic potential in the adult brain.
In contrast to this view, comparative studies showed that in some mammalian species, including humans, low levels of neurogenesis can also occur in regions normally non-neurogenic in mice, such as the striatum and neocortex. Further, brain lesions can induce neurogenesis in these same regions also in laboratory rodents. In the striatum, the origin of part of the newly generated neurons could be traced to local parenchymal astrocytes. These results revealed the presence of a latent NSC potential outside of the canonical niches. However, the prevalence, distribution, behavior and cell fate potential of these ectopic NSCs have not been established. Consequently, the extent to which the neurogenic potential of the mature brain parenchyma differs from that of conventional neurogenic niches remains to be determined.

To fill these gaps, we focused on a mouse model of striatal neurogenesis induced intra-striatal infusion of quinolinic acid (QA) that causes an excitotoxic lesion. In this model a huge number of immature neuroblasts is generated for several months exclusively from local astrocytes. Following excitotoxic injury, striatal astrocytes spontaneously activate this potential at the lesion border, displaying similar spatiotemporal dynamics as observed in canonical niches. Our data indicate that the prevalence of neurogenic astrocytes in the striatal parenchymal is similar to that in the SVZ niche. Striatal astrocytes neurogenic activation leads to the continuous and widespread generation of LGE-class interneurons resembling those produced in the adult V-SVZ and during the perinatal period in various brain regions. Remarkably, despite their transient nature, newly generated striatal neurons functionally integrate into brain circuits, suggesting a potential plastic role in post-lesion circuit reorganization.
These findings challenge the notion that NSCs are rare cells confined to specific regions in the adult brain. Additionally, contrary to previous beliefs, the brain parenchyma is largely conducive to the maintenance and activation of NSCs, similar to canonical niches. Notably, adult V-SVZ NSCs and parenchymal astrocytes

share a common cell fate potential. However, whether the neurons generated by parenchymal astrocytes play a role in post-lesion recovery deserve further investigation.

Friday 22/11/2024 h. 3.00 pm - Webinar

Bilal E. Kerman, Keck School of Medicine of USC - University of Southern California

Exploring APOE4's Role in Alzheimer's Disease Using Stem Cells

Human Apolipoprotein (APOE) has three isoforms, *ε2*, *ε3*, and *ε4* among which *ε4* (*APOE4*) confers the highest risk for late-onset Alzheimer's disease (AD). APOE4 is also the most prone to aggregate among APOE isoforms. Current evidence strongly suggests that APOE aggregation leads to neuronal dysfunction and eventually to AD. APOE4 increases amyloid plaques and neurofibrillary tangles and decreases synapses and neuronal survival. These phenotypes are alleviated by decreasing APOE4 aggregation. First, we analyzed APOE aggregation using fluorescence lifetime imaging microscopy (FLIM) in combination with Forster resonance energy transfer (FRET). APOE aggregation was also confirmed by using small-angle X-ray scattering (SAXS) of the Sarkosyl extracts of the cells.APOE4 aggregated more than APOE3 in living cells. Additionally, lipidation decreased its aggregation in line with published data. Then, secreted APOE-Tdtomato was isolated and shown to be in HDL particles by ion mobility assay. Human neurons endocytosed APOE-Tdtomato. APOE4 recycled less to the membrane and was prone to degradation as shown by detailed image analysis. These observations suggest that APOE4 aggregated more in human neurons and impaired intracellular trafficking.

Teaser: Label-free holotomographic imaging can visualize myelinated axons and quantify g-ratios.

Host: Giovanna Gambarotta

Friday 29/11/2024 h. 2.00 pm - Hybrid seminar

Silvia Giatti, Unit of Neuroendocrinology, University of Milan

New insights into the post-SSRI sexual dysfunction (PSSD) syndrome

Antidepressants are a widely prescribed class of drugs used to treat mood disorders such as depression, anxiety, premenstrual dysphoric disorder, and post-traumatic stress disorder. Among the various types of antidepressants, selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) are the most commonly used. Despite their effectiveness, these medications are often associated with side effects, with sexual dysfunction being one of the most frequently reported issues. This side effect can negatively impact patients' adherence to therapy and reduce overall quality of life, leading to treatment discontinuation in some patients.

Notably, some patients continue to experience sexual dysfunction even after discontinuing SSRIs/SNRIs, or they may develop new sexual issues after discontinuingthe drug. This condition is known as post-SSRI sexual dysfunction (PSSD), a poorly understood disorder with an unknown cause and no established treatment.

To address the challenges of PSSD, we developed an animal model to study the condition. Previous research has shown that SSRI treatment in rodents can impair sexual function, mirroring the effects observed in humans. In our lab, we use this well-established model to investigate sexual dysfunction following SSRI administration, examining not only the immediate effects during treatment but also the long-term impact after a one-month withdrawal period. Our published data suggest that neuroactive steroids—molecules that play key roles in regulating brain function, including sexual behavior—are involved in PSSD. Analysis of brain regions from our animal models revealed altered neuroactive steroid levels after SSRI treatment and after drug withdrawal[15]. Moreover, the gut microbiota component seems to be altered by paroxetine treatment and suspension[16]. Additionally, recent RNA sequencing experiments have identified modifications in brain pathways related to sexual function, potentially revealing mechanisms that contribute to persistent sexual dysfunction in PSSD (under review - Molecular Neurobiology).

Host: Marilena Marraudino

Friday 6/12/2024 h. 2.00 pm - Hybrid seminar

Elena Giglia, University of Turin and Open Science

Open Science why and how

How does Open Access and Open Science relate? And what about EOSC and FAIR data? During this seminar we shall explore not only the European requirements on Open and FAIR science but also the reasons why we need data and results “as open as possible” for a better science, more transparent, effective and sound.

Host: Serena Bovetti

Friday 20/12/2024 h. 10.00 am - Hybrid seminar

Gabriele Chelini, CNR Pisa

Focal cluster of peri-synaptic matrix contribute to activity-dependent plasticity and memory in mice

Recent findings show that effective integration of novel information in the brain requires coordinated processes of homo- and heterosynaptic plasticity. In this work, we hypothesize that activity-dependent remodeling of the peri-synaptic extracellular matrix (ECM) contributes to these processes. We show that clusters of the peri-synaptic ECM, recognized by CS56 antibody, emerge in response to sensory stimuli, showing temporal and spatial coincidence with dendritic spine plasticity. Using CS56 co-immunoprecipitation of synaptosomal proteins, we identify several molecules involved in Ca2+ signaling, vesicle cycling, and AMPA-receptor exocytosis, thus suggesting a role in long-term potentiation (LTP). Finally, we show that, in the CA1 hippocampal region, the attenuation of CS56 glycoepitopes, through the depletion of versican as one of its main carriers, impairs LTP and object location memory in mice. These findings show that activity-dependent remodeling of the peri-synaptic ECM regulates the induction and consolidation of LTP, contributing to hippocampal-dependent memory.

Host: Ilaria Bertocchi