New perspectives in Neuroscience: Research Results of Young Italian Neuroscientists

National Meeting of PhD students in Neuroscience
June 11, 2022
Brescia

FULL PROGRAM
PATRONAGE AND SPONSORS

SINS

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GENERAL INFORMATION

Venue:
The National Meeting of PhD students in Neuroscience will be held at the “Aula Magna – Facoltà di Medicina”, Università degli Studi di Brescia, Viale Europa 11, 25123, Brescia.

How to reach us?
Brescia is located in Lombardy region, Italy, between Milan and Venice.

The “Aula Magna – Facoltà di Medicina”, Università degli Studi di Brescia, is located in the north of Brescia. You can easily reach it by underground (metro). From Brescia railway station “Stazione FS” underground station or from anywhere in the city center, take the Northbound line (end-of-line station “Prealpino”) and get off at “Europa” underground station (https://www.bresciamobilita.it/).

The University of Brescia, Medicine Campus is just 300 m away along Viale Europa.

Download Bresciapp! on your mobile to more information

You can reach the Medicine Campus by bus or by car or taxi, as well!

By plane
There are three airports close to Brescia in the range of 50 Km:

- Bergamo – Orio al Serio airport (BGY)
  Reach Brescia by shuttle bus to get from Bergamo – Orio al Serio Airport directly to Brescia railway station.

- Verona – Villafranca airport (VRN)
  Reach Brescia by train from Verona Porta Nuova railway station, you can take a train to Brescia railway station (travel time of about 45 min).

- Milan – Linate airport (LIN)
  Reach Brescia by train. From Milan – Linate airport, you can reach Milan Central railway station (“Stazione Milano Centrale”) by taxi (a 10 min ride) or by bus. You can then take a train to Brescia and get to Brescia railway station (travel time of about 50 min).

Registration and secretariat desk:
Registration desk will be open from 07:15 am during the congress, and closed at the end of the meeting.

Poster Presentations:
Please check the assigned number to in order to hang the poster in the correct assigned space. Poster will be hold for the entire meeting and removed before the end of the day. The organizer are not responsible for anything left unattended at the poster site, including posters.
## PROGRAM

**11 June 2022**

### 8:00 – 8:15  
**Introduction to the Meeting by Organizers and SINS**

*President SINS Prof. Alessandro Vercelli*

### 8:15 – 9:45  
**SYMPOSIUM 1: Oral Presentations**

*Chairman: Buonincontri V., University of Campania*  
*Parente M., University Roma Tre*

<table>
<thead>
<tr>
<th>Time</th>
<th>Title</th>
<th>Speaker</th>
<th>Institution</th>
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<tbody>
<tr>
<td>8:15</td>
<td>Primary sensory cortices of a mouse model of CDKL5 deficiency disorder show atypical myelination</td>
<td>Devi S., University of Torino</td>
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<tr>
<td>8:30</td>
<td>Characterization of the extracellular vesicles released by astrocytes cultured from the spinal cord of 120-day-old SOD1&lt;sup&gt;G93A&lt;/sup&gt; mice</td>
<td>Zerbo A., University of Genova</td>
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<td>8:45</td>
<td>Is alpha-synuclein a foe for brain recovery after ischemic stroke?</td>
<td>Bogale T.A., University of Brescia</td>
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<tr>
<td>9:00</td>
<td>Targeting the endocannabinoid and melatonergic systems to provide neuroprotection against the neuroinflammatory damage</td>
<td>Cammarota M., University of Napoli Federico II</td>
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<tr>
<td>9:15</td>
<td>Somatosensory processing deficits and altered connectivity in Cntnap2&lt;sup&gt;-&lt;/sup&gt;/Shank3b&lt;sup&gt;-&lt;/sup&gt; mouse models of autism spectrum disorder</td>
<td>Balasco L., University of Trento</td>
<td></td>
</tr>
<tr>
<td>9:30</td>
<td>Alterations in extracellular spaces thickness and dopaminergic activity in CKD mice</td>
<td>De Donato A., University of Campania</td>
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### 9:45 – 10:30  
**Lecture 1:** “A modular strategy for next-generation upper-limb sensory-motor neuroprostheses”

*Dr. Solaiman Shokur, EPFL, Geneva, Switzerland*

*Chair: Prof. Viviana Trezza*

### 10:30 – 10:45  
**Coffee Break**
10:45 – 12:25 SHORT ORAL PRESENTATIONS

Chairman: Nappi M., University of Napoli
Preziuso A., University Politecnica delle Marche

10:45 – 10:55 Semaphorin-3A drives axonal growth cone elongation during neuronal development
Ferretti G., University of Napoli Federico II

10:55 – 11:05 Mind the gap: a role for ER-mitochondria interaction, in Alzheimer’s disease astrocytes cellular dysfunction
De Matteis G., University of Piemonte Orientale

11:05 – 11:15 In vivo neurotransmitter deficits in genetic frontotemporal dementia. a GENFI study
Pengo M., University of Brescia

11:15 – 11:25 Engineering LIM kinase 1 to control dendritic spine plasticity
Sollazzo R., IRCCS Policlinico Gemelli

11:25 – 11:35 Rescuing neural cell survival and maturation in a primary autosomal recessive microcephaly-17 (MCPH17) mouse model: effects of the postnatal N-acetyl cysteine
Khastkhodaei Ardakani M., University of Torino

11:35 – 11:45 The acute effects of intrusive thinking on neurotransmission within anterior cingulate cortex in pathological and non-pathological worriers: A 1H magnetic resonance spectroscopy and ecological momentary assessment study.
Schettino M., Sapienza University of Rome

11:45 – 11:55 Insights into the neurodegenerative mechanisms associated with KIF5A mutations
Cozzi M., University of Milano

11:55 – 12:05 Synaptic targets of KLVFF: the impact on the neurotransmitters release
Trebesova H., University of Genova

12:05 – 12:15 Insight into Frontotemporal Dementia pathogenesis: the role of anti-GluA3 antibodies
Italia M., University of Milano

12:15 – 12:25 Long-term effect after intravenous self-administration (IVSA) of the synthetic cannabinoid receptor agonist 5F-MDMB-PICA in adolescent mice
Caria F., University of Cagliari

12:25 – 14:15 Lunch and POSTER SECTION

Posters evaluated by the members of the SINS Board
**14:15 – 15:00**  
**Lecture 2:** “Of life and death of neurons”  
Prof. Alessandro Vercelli, SINS President  
*Chair: Prof. Marina Pizzi*

**15:00 – 17:15**  
**SYMPOSIUM 2: Oral Presentations**  
*Chairman: Tomasoni Z., University of Brescia*  
*Camoglio C., University of Cagliari*

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<tr>
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<th>Presenter</th>
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<tr>
<td>15:00 – 15:15</td>
<td>Cognitive decline induced by intracerebral infusion of human-alpha synuclein oligomers is associated with altered neuronal firing in cingulate cortical neurons</td>
<td>Palmas F., University of Cagliari</td>
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<td>15:30 – 15:45</td>
<td>Mitochondrial alterations in subjects with idiopathic REM sleep disorders as a predictive biomarker for conversion to Parkinson’s disease</td>
<td>Ongari G., IRCCS Mondino Foundation</td>
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<td>15:45 – 16:00</td>
<td>Extracellular vesicles cooperate with PQC system for the clearance of TDP-43 species associated with ALS and FTD</td>
<td>Casarotto E., University of Milano</td>
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<tr>
<td>16:00 – 16:15</td>
<td>Evidence of vestibular contributions to visuospatial attention in patients with vestibular disorders</td>
<td>Gammeri R., University of Torino</td>
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<tr>
<td>16:15 – 16:30</td>
<td>Improved synapsins phosphorylation and alpha-synuclein nitration occur in concert in experimental models of Parkinson’s disease</td>
<td>Brembati V., University of Brescia</td>
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<tr>
<td>16:30 – 16:45</td>
<td>Beyond retigabine: design, synthesis, and pharmacological characterization of a potent and chemically-stable neuronal Kv7 channels activator with anticonvulsant activity</td>
<td>Carotenuto L., University of Napoli Federico II</td>
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<tr>
<td>16:45 – 17:00</td>
<td>Functional effects of a new dual adenosine A2A/A2B receptor antagonist on CA1 hippocampal synaptic plasticity or during oxygen glucose deprivation</td>
<td>Venturini M., University of Firenze</td>
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<tr>
<td>17:00 – 17:15</td>
<td>Dissecting the neural bases behind observational learning of complex social behaviors</td>
<td>La Greca F., University of Milano</td>
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**17.15 – 17.45**  
**Poster Awards, Conclusions and Final Remarks**
POSTER INDEX
POSTER SESSION
Disorders of the nervous system

P01. Effect of neonatal hypoxic-ischemic brain injury on rat neural stem cells differentiation: ex vivo and in vitro studies
Alastra G.1, Baldassarro V. A.1,3, Ferrazzi F.2, Sannia M.3, Calzà L.2,3,4
1Department of Veterinary Medical Sciences, University of Bologna, Italy; 2IRET Foundation, Ozzano Emilia, Italy; 3Health Science and Technologies Interdepartmental Center for Industrial Research (HST-ICIR), University of Bologna, Italy; 4Department of Pharmacy and Biotechnology, University of Bologna, Italy

P02. Investigating the role of large microglial extracellular vesicles carrying pathogenic misfolded proteins in Alzheimer’s disease and their interaction with neurons
Battocchio E.1,2, Gabrielli M.1, Verderio C.1
1CNR Institute of Neuroscience, Vedano al Lambro, MB, 20854, Italy; 2School of Medicine and Surgery, Department of Medicine and Surgery, University of Milano-Bicocca, Monza, 20900, Italy

P03. Selective behavioral alterations after acute particulate matter exposure in a pre-symptomatic Multiple Sclerosis mouse model
Bonato M.1, Montarolo F.2,3, Parolisi R.1, Pandino C.1, Bertolotto A.2, Buffo A.1, Boda E.1
1Dept. of Neuroscience, Università degli Studi di Torino, Torino, Italy; 2Neuroscience Institute Cavalieri Ottolenghi, Orbassano (Torino), Italy; 3Neurobiology Unit, Neurology-CReSM (Regional Referring Center of Multiple Sclerosis), AOU San Luigi Gonzaga, Orbassano (Torino), Italy

P04. Opposite effects of chronic kidney disease and mild cognitive impairment on the diffusion of water within the perivascular space
Buonincontri V.1, Viggiano D.1,2
1Dipartimento di Salute Mentale, fisica e Medicina Preventiva, Università degli studi della Campania “Luigi Vanvitelli”, Napoli; 2Dipartimento di Scienze Mediche Traslazionali, Università degli studi della Campania “Luigi Vanvitelli”, Napoli

P05. Evaluation of Neuroinflammatory Markers in an animal model of Anorexia Nervosa
Camoglio C.1, Scherma M.1, D’Amelio S.2, Spero V.2, Molteni R.2, Fadda P.1
1Department of Biomedical Sciences, Division of Neuroscience and Clinical Pharmacology, University of Cagliari, Italy; 2Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy

P06. Investigation of mitochondrial SMN1-anticorrelated genes as possible therapeutic targets for Spinal Muscular Atrophy
Caretto A.1,2, Di Cunto F.1,2, Boido M.1,2, Vercelli A.1,2
1Department of Neuroscience Rita Levi Montalcini, University of Turin, 10126 Turin, Italy; 2Neuroscience Institute Cavalieri Ottolenghi, University of Turin, 10043 Orbassano (TO), Italy

P07. The reversible Carnitine Palmitoyltransferase1 inhibitor ST1326 ameliorates the neurodegenerative phenotype in a Drosophila Huntington model by acting on the expression of carnitine-related genes
Carillo M. R., Bertapelle C., Peluso G., Digilio F.A
National Research Council, Napoli, Italy and University of Campania “Luigi Vanvitelli”

P08. Curcumin prevents oxidative stress induced alteration on ARPE-19 cells
Carozza G.1, Tisi A.1, Flati V.1, Feligioni M.2, Maccarone R.1
1Department of Biotechnological and Applied Clinical Sciences, University of L’Aquila, 67100-L’Aquila; 2European Brain Research Institute, 00161 Rome, Italy
P09. Survival of VTA dopaminergic neurons is associated with overexpression of Ca^{2+}-binding proteins in the Tg2576 mouse model of Alzheimer's Disease

Cauzzi E.
Laboratory of Molecular Neuroscience, Campus Bio-medico University of Rome, Italy; Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy; Institute of Biochemistry and Cell Biology (IBBC) - National Research Council (CNR), Rome, Italy

P10. Alternative Translation Initiation as a novel strategy to block toxicity of the mutant Androgen Receptor in SBMA

Chierichetti M.1, Cristofani R.1, Rusmini P.1, Ferrari V.1, Tedesco B.1, Cozzi M.1, Casarotto E.1, Mina F.1, Pramaggiore P.1, Crippa V.1, Galbiati M.1, Piccolella M.1, Poletti A.1
1Dipartimento di Scienze Farmacologiche e Biomolecolari-Centre of Excellence on Neurodegenerative Diseases, Università degli Studi di Milano, Italy

P11. Synaptic alterations in the auditory cortex and hippocampus underlie social deficits in the Synapsin II knockout mouse

Ciano L.1,2, Esposito A.1,2, Franchi F.1,2, Fassio A.2,3, Michetti C.1,2, Benfenati F.1,3
1Center for Synaptic Neuroscience and Technology, Italian Institute of Technology, Genova, Italy; 2Department of Experimental Medicine, University of Genova, Genova, Italy; 3IRCCS, Policlinico San Martino Hospital, Genova, Italy

P12. Epigenetic and transcriptional perturbations underlie defective myelooarchitecture in the visual cortex of CDKL5-deficiency disorder patients

Comai D.1, Pizzo R.1, Devi S.1, Lauria A.2,3, Anselmi F.2,3, Gurgone A.1, Oliviero S.2,3, Giustetto M.1
1Department of Neuroscience, University of Turin, Turin (Italy); 2Department of Life Sciences and System Biology and MBC, University of Turin, Turin (Italy); 3IIGM - Italian Institute for Genomic Medicine, Candiolo (Italy)

P13. Morphological characterization of adult-born hippocampal neurons in a mouse model of the neurodevelopmental disorder bbsosa

Dallorto E.1,2, Bonzano S.1,2, Pattaro A.1,2, Studer M.3, De Marchis S.1,2
1Neuroscience Institute Cavalieri Ottolenghi (NICO), Orbassano (Turin), Italy; 2Department of Life Sciences and Systems Biology (DBIOS), University of Turin, Italy; 3Institute of Biology Valrose (IBV), Univ. Côte d'Azur, CNRS, Inserm, Nice, France

P14. Effect of the homeobox gene Dbx2 on astrocyte functional changes and their consequence on the regulation of neural stem cells properties

D’Angelo S.1, Cortese P.1, Lupo G.1, Cacci E.1
1Dipartimento di Biologia e Biotecnologie “C. Darwin”, Università di Roma La Sapienza

P15. Chemogenetic manipulations of hippocampus excitability in Ambra1+/− mice with sexual dimorphism of autistic traits: implications for female autism

De Introna M.1,2,3, Sabetta A.3, Nobili A.3,4, Stabile F.3, D’Addario S. L.3,5, Ventura R.3,5, Ammassari-Teule M.3,6, Pignataro A.2,3
1Department of Systems Medicine, Tor Vergata University, Rome, Italy; 2Institute of Translational Pharmacology, CNR-National Research Council, Rome, Italy; 3IRCCS Santa Lucia Foundation (FSL), Centro di Ricerca Europeo sul Cervello (CERC), Rome, Italy; 4University Campus Bio-Medico, Rome, Italy; 5Department of Psychology, University Sapienza, Rome, Italy; 6Department of Biochemistry and Cell Biology, CNR National Research Council, Rome, Italy

P16. Correlation between D-loop methylation level and mtDNA copy number in Aicardi-Goutières patients

Dragoni F.1,2, Garau J.1, Orcesi S.1, Pansarasa O.1, Gagliardi S.1
1IRCCS Mondino Foundation, Pavia, Italy; 2Department of Biology and Biotechnology “Lazzaro Spallanzani”, University of Pavia, Pavia, Italy
P17. Increased DUX4 expression and correlation with TDP-43 in peripheral cells from ALS patients
Duranti E.1,2, Sala G.1, D’Orlando C.1, Gerardi F.1, Riva N.1, Lunetta C.2, Meneveri R.1, Ferrarese C.1,4, Tremolizzo L.1,4
1School of Medicine and Surgery and Milan Center for Neuroscience (NeuroMI), University of Milano-Bicocca, Monza; 2NeuroMuscular Omnicentre (NEMO), Fondazione Onlus, Milano; 3Neurology Unit, IRCCS San Raffaele Scientific Institute Milano; 4Department of Neurology, ASST Monza, San Gerardo Hospital, Monza; 5PhD program in Neuroscience, University of Milano-Bicocca, Monza

P18. Dopamine signaling in striatal astrocytes
Favetta G.1, Masato A.1, Bubacco L.1
1Unit of Molecular and Cellular Physiology and Biophysics, Department of Biology, University of Padova, Italy

P19. Adenosine A2B receptor activation regulates oligodendroglial differentiation and myelination: an in vitro study
Frulloni L.1, Santalmasi C.1, Cherchi F.1, Venturini M.1, Magni G.2, Rossi F.2, Pedata F.1, Cencetti F.3, Coppi E.1, Pugliese A.M.1
1Department of Neuroscience, Psychology, Drug Research and Child Health, NEUROFARBA, Section of Pharmacology and Toxicology, University of Florence, Florence, Italy; 2Istituto di Fisica Applicata, CNR, Via Madonna del Piano 10, Sesto Fiorentino 50019, Italy; 3Department of Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy

P20. Deficient NF-kB/c-Rel activity in pathophysiology of Parkinson’s disease
Gennari M.M.1, Porrini V.1, Parrella E.1, Gussago C.1, Pilotto A2, Vezzoli M.1, Bellucci A.1, Padovani A.2, Pizzi M.1
1Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy; 2Neurology Unit, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy

P21. New evidence of a different activation of astrocytes in LPS and MCAO gliosis mice model: a computational transcriptome analysis
Gioia C.1,2, Goglia I.1,3, Martorana F.1, Bertolazzi P.4, Colangelo A.M.1
1Laboratory of Neuroscience “R. Levi-Montalcini”, Dept. of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy; 2PhD course in Translational and Molecular Medicine (DIMET), University of Milano-Bicocca, Milan, Italy; 3PhD program in Neuroscience, School of Medicine and surgery, University of Milano-Bicocca, Milan, Italy; 4Institute of Systems Analysis and Computer Science A. Ruberti (IASI), National Research Council (CNR), Via dei Taurini 19, 00185 Rome, Italy

P22. Generation and characterization of the c19orf12 mutant zebrafish model
Gnutti B., Agazzi P., Mignani L., Canino B.F., Zizioli D., Borsani G., Finazzi D.
Department of Molecular and Translational Medicine, University of Brescia, Italy

P23. Live imaging of cell motility and mitochondrial dynamics provides a new energy-consuming mechanism required for NGF-induced differentiation
Goglia I.1,2, Gioia C.1,3, Martorana F.1, Colangelo A.M.1
1Laboratory of Neuroscience “R. Levi-Montalcini”, Dept. of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy; 2PhD Program in Neuroscience, School of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy; 3PhD Course in Translational and molecular medicine (DIMET), University of Milano-Bicocca, Milan, Italy
P24. Development of Neural Stem Cell-based experimental therapy for the treatment of Amyotrophic Lateral Sclerosis


1Department of Biotechnology and Biosciences, University of Milano-Bicocca; 2Fondazione IRCCS, Casa Sollievo della Sofferenza; 3UPTA Unit, Fondazione IRCCS Casa Sollievo della Sofferenza; 4Neuroscience Institute Cavalieri Ottolenghi, Department of Neuroscience “Rita Levi Montalcini”, University of Turin.

P25. Effects of dopaminergic medication on reactive and proactive inhibitory control


1Department of Clinical and Experimental Sciences, University of Brescia, Viale Europa 11, 25123 Brescia (BS), Italy; 2IRCCS Neuromed, Via Atinense 18, 86077 Pozzilli (IS), Italy

P26. Generation and characterization of ap1s2 mutant lines in *Danio rerio*

**Massardi E.**, Facchinello N., Mignani L., Finazzi D., Monti E., Zizioli D., Borsani G.

1Division of Biology and Genetics, Department of Molecular and Translational Medicine, University of Brescia, Italy; 2Department of Molecular Medicine, University of Padua, Italy; 3Division of Biotechnology, Department of Molecular and Translational Medicine, University of Brescia, Italy

P27. Gain-of-function due to increased opening probability by two KCNQ5 pore variants causing developmental and epileptic encephalopathy


1Department of Neuroscience, University of Naples “Federico II”, 80131 Naples, Italy; 2Department of Medical Genetics Lyon University Hospital, Claude Bernard Lyon 1 University, 69677 Lyon, France; 3Department of Pediatric Neurology, Lyon University Hospital, Claude Bernard Lyon 1 University, 69677 Lyon, France; 4CHU Lille, Institut de Génétique Médicale et Univ. Lille, EA 7364 – RADEME - Maladies Rares du Développement embryonnaire et du MÉtabolisme, F-59000 Lille, France; 5CHU Lille, Clinique de Génétique – Guy Fontaine, F-59000 Lille, France; 6Department of Pediatrics, Hôpital Nord-Ouest, Villefranche-sur-Saône, 69400, France; 7Department of Medicine and Health Science, University of Molise, 86100 Campobasso, Italy; 8Department of Science and Technology, University of Sannio, 82100 Benevento, Italy; 9Institute of Biophysics, Italian National Research Council, 16149 Genova, Italy

P28. AMPA and NMDA receptors expression modifications in the PFC of rat model of PTSD and after ketamine treatment

**Ndjo E.**, Mingardi J., Carini G., La Via L., Musazzi L., Barbón A.

1Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy; 2School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy

P29. Repurposing pomalidomide as a neuroprotective drug in an alpha-synuclein-based model of Parkinson’s disease


1Department of Biomedical Sciences, University of Cagliari, Italy; 2Department of Biomedical and Biotechnological Sciences, University of Catania, Italy; 3CNR Institute of Translational Pharmacology, Cagliari, Italy; 4Department of Pharmacy, University of Naples “Federico II”, Italy; 5Centre for Misfolding Diseases, Department of Chemistry, University of Cambridge, Cambridge, UK; 6Department of Life and Environmental Sciences, University of Cagliari, Italy; 7Drug Design & Development Section, Translational Gerontology Branch, Intramural Research Program, National Institute on Aging, National Institutes of Health, Baltimore, MD, United States; 8National Research Council, Institute of Neuroscience, Cagliari, Italy.
P30. BET proteins: a new therapeutic target for Niemann-Pick type C1 disease?
Parente M., Tonini C., Pallottini V.
Department of Science, University Roma Tre, Viale Marconi 446, 00146, Rome

P31. Cannabidiol promotes social behavior in mice: study on mechanism of action
Premoli M., Bonini S. A., Mastinu A., Maccarini G., Memo M.
Section of Pharmacology, Department of Molecular and Translational Medicine, University of Brescia, Italy

P32. New antioxidant K+ channel-independent effect of XE-991 in an in vitro model of metabolic impairment: implications for Alzheimer’s Disease
Preziuso A., Piccirillo S., Amoroso S., Serfilippi T., Miceli F., Magi S., Lariccia V.
1Department of Biomedical Sciences and Public Health, School of Medicine, University “Politecnica delle Marche”, Via Tronto 10/A, 60126, Ancona, Italy; 2Department of Neuroscience, University of Naples, “Federico II”, Via Pansini 5, 80131, Naples, Italy.

P33. Unravelling the effect of stressors on Amyotrophic Lateral Sclerosis onset and progression: preliminary in vitro experiments
Rasà D., Boido M.
Department of Neuroscience Rita Levi Montalcini, Neuroscience Institute Cavalieri Ottolenghi, University of Turin, Italy.

P34. Human iPSCs-derived oligodendrocytes and astrocytes as the first Autosomal Dominant Leukodystrophy-relevant cellular models
Ribodino M., Lorenzati M., Signorino E., Nicorvo E., Grimaldi P., Conti L., Cortelli P., Berchialla P., Giorgio E., Buffo A.
1University of Turin, Department of Neuroscience “Rita Levi Montalcini”, Turin, Italy; 2Neuroscience Institute Cavalieri Ottolenghi (NICO), Orbassano, Italy; 3Department of Sciences of Public Health and Pediatrics, University of Turin, Turin, Italy; 4University of Trento, Centre for Integrative Biology (CIBIO), Laboratory of Computational Oncology, Trento, Italy; 5IRCCS Istituto delle Scienze Neurologiche di Bologna, Bellaria Hospital, Bologna, Italy; 6University of Bologna, Department of Biomedical and Neuromotor Sciences, Bologna, Italy; 7Department of Clinical and Biological Sciences, University of Torino, Orbassano, Italy; 8University of Pavia, Department of Molecular Medicine, Pavia, Italy; 9IRCCS Mondino Foundation, Laboratory of Molecular Medicine and Cytogenetics, Pavia, Italy

P35. Motor neural organoids for the study of ALS
Scarian E., Bordoni M., Garofalo M., Gagliardi S., Pansarasa O.
1Department of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy; 2IRCCS Mondino Foundation, Pavia, Italy

P36. Morpho-functional alterations in epithelial cells of the Choroid Plexus during aging
Scarpetta V., Bodaleo Torres F., Salio C., Sassoé-Pognetto M., Agarwal A.2, Patrizi A.
1Department of Neurosciences, University of Turin, Turin, Italy; 2Chica and Heinz Schaller Research Group, Institute for Anatomy and Cell Biology, Heidelberg, Germany; 3Department of Veterinary Sciences, University of Turin, Turin, Italy; 4Schaller Research Group, German Cancer Research Center (DKFZ), Heidelberg, Germany

P37. Inflammatory pathway dysregulations promote amyloid beta precursor protein phosphorylation on Tyr682 residue in monocytes from patients with Alzheimer’s disease
Serafini S., Ferreti G., Angiollillo A., Di Costanzo A.2, Frisardi V., Maier J T., and Matrone C.
1Unit of Pharmacology, Department of Neuroscience, Faculty of Medicine, University of Naples Federico II, Naples, Italy; 2Center for Research and Training in Medicine of Aging, Department of Medicine and Health Sciences, University of Molise, Via F. De Sanctis 1, 86100, Campobasso, Italy; 3Paul-Ehrlich Institute, Federal Institute for Vaccines and Biomedicines, Langen, Germany

P38. The cyclase-associated protein 2 controls Cofilin-actin rods formation in Alzheimer’s Disease
Stringhi R., D’Andrea L., Vandermeulen L., Ascagni M.2, Di Luca M., Pelucchi S., Marcello E.
1Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan, Italy; 2Unitech NOLIMITS, Università degli Studi di Milano, Milan, Italy
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Tessitore S.1, Torazza C.1, Kumar M.1, Allan S.2, Shaw P. J.2, Ferraiuolo L.2, Bonanno G.1,3, Milanese M.1

1University of Genoa, Department of Pharmacy-Pharmacology and Toxicology Unit, Genoa, Italy; 2University of Sheffield, Sheffield Institute of Translational Neuroscience (SiTraN), Sheffield, United Kingdom; 3IRCCS, Ospedale Policlinico San Martino, Genoa, Italy.

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1Division of Pharmacology, Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy; 2Division of Biology and Genetics, Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy; 3Genetic Unit, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy

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1Section of Pharmacology, Department of Molecular and Translational Medicine, University of Brescia, Viale Europa, 11, 25123, Brescia (Italy); 2Section of Biology and Genetics, Department of Molecular and Translational Medicine, University of Brescia, Viale Europa, 11, 25123, Brescia (Italy); 3Section of Biotechnologies, Department of Molecular and Translational Medicine, University of Brescia, Viale Europa, 11, 25123, Brescia (Italy)
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Disorders of the nervous system
Primary sensory cortices of a mouse model of CDKL5 deficiency disorder show atypical myelination.

Sunaina Devi¹, Debora Comai¹, Riccardo Pizzo¹, Antonia Gurgone¹, Martina Lorenzati¹,² Annalisa Buffo¹,², Chiara Salio³ and Maurizio Giustetto¹

¹Department of Neuroscience, University of Turin, Turin (Italy); ²Neuroscience Institute Cavalieri Ottolenghi (NICO), University of Torino, ³Department of Veterinary Science, University of Turin Grugliasco, TO, Italy.

**Introduction and objectives:** CDKL5 deficiency disorder (CDD) is a rare neurodevelopmental condition without a cure caused by mutations in cyclin-dependent kinase-like 5 (CDKL5) gene, characterized by early-onset epilepsy, severe cognitive dysfunctions, sensorimotor and intellectual disabilities (Archer et al., 2006). CDKL5 is a serine/threonine kinase expressed early during postnatal development in neurons where it phosphorylates epigenetic factors, elements of both axonal and dendritic compartment, and microtubule-associated proteins which are crucial in nucleation and assembly of microtubules (Barbiero et al., 2019). Along with neurons, CDKL5 is also expressed in oligodendroglia, underlying the myelination process (Zhang et al., 2016, 2014). Although growing evidence indicates that the organization of myelin sheath is often severely compromised in neurodevelopmental diseases (Zhao et al., 2018), whether CDKL5 mutation affects myelin is still completely unknown.

**Results:** To start addressing this issue, we evaluated both organization and developmental trajectory of myelination by analyzing the expression of molecules modified by myelin deposition or axonal injury—i.e.: Myelin basic protein (MBP) and neurofilaments (NF)—in both young (PND15) and adult (PND56) Cdkl5-KO mice. This analysis showed a severe reduction of both markers expression in primary sensory cortices of Cdkl5-KO mice. The g-ratio analysis investigated by electron-microscopy revealed that average myelin sheath thickness is decreased in mutant mice. Intriguingly, the analyses of of nodes of Ranvier, demonstrated alterations in length and density of nodal/paranodal compartments in Cdkl5-KO mice. Finally, the density of mature oligodendrocytes was reduced in mutants whereas oligodendrocyte precursor cells number was apparently not affected.

**Conclusions:** Our data indicate that primary cortical areas in CDD animals exhibit a severe reduction/distortion of the myelination process and disclose a novel role of CDKL5 activity, likely of pivotal importance for CDD.
Characterization of the extracellular vesicles released by astrocytes cultured from the spinal cord of 120-day-old SOD1\textsuperscript{G93A} mice

Roberta Arianna Zerbo\textsuperscript{1}, Fabrizio Fabbiano\textsuperscript{2}, Carola Torazza\textsuperscript{1}, Sara Tessitore\textsuperscript{1}, Marco Milanese\textsuperscript{1,3}, Vito G. D’Agostino\textsuperscript{2*}, Giambattista Bonanno\textsuperscript{1,4*}.

*equally contributed

\textsuperscript{1} Department of Pharmacy, Pharmacology and Toxicology Unit, University of Genoa, Genoa, Italy; \textsuperscript{2} Department of Cellular, Computational and Integrative Biology, University of Trento, Trento, Italy; \textsuperscript{3} Inter-University Center for the Promotion of the 3Rs Principles in Teaching & Research (Centro 3R), Genoa, Italy; \textsuperscript{4} IRCCS, Ospedale Policlinico San Martino, Genoa, Italy.

Introduction and objectives: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that affects upper and lower motor neurons (MNs), leading to muscle atrophy, paralysis, and death within 3-5 years from symptom onset. ALS is a non-cell-autonomous disease in which astrocytes play a crucial role in MNs damage and death, possibly by secretion of soluble factors or extracellular vesicles (EVs). This study will reveal the part played by EVs produced by astrocytes from adult, late symptomatic, SOD1\textsuperscript{G93A} mice, an ALS animal model, in the astrocyte and MN interplay in ALS.

Results: Astrocytes were cultured (20 DIV) from the spinal cord of 120-day-old SOD1\textsuperscript{G93A} and WT mice. EVs were isolated by the nickel-based isolation (NBI) procedure from the astrocyte supernatant. The number of cells obtained from SOD1\textsuperscript{G93A} and WT mice was 6.37x10\textsuperscript{5} and 1.61x10\textsuperscript{5}, respectively. EVs recovered per cell was 6.73x10\textsuperscript{5} from SOD1\textsuperscript{G93A} astrocytes and 1.78x10\textsuperscript{6} from WT astrocytes. We did not detect size or Z potential differences between the two EVs genotypes. We now completed the RNA sequencing to define the microRNA repertoire of SOD1\textsuperscript{G93A} and WT astrocyte-derived EVs. Bioinformatic analysis is ongoing. Exposure to EVs from SOD1\textsuperscript{G93A} astrocytes significantly reduced MN viability compared to controls.

Conclusions: Our results indicate that pathological astrocytes influence MN viability in the SOD1\textsuperscript{G93A} mouse model of ALS through EVs secretion and suggest that they can carry unique information that defines their neurotoxic activity. Data from RNA sequencing should shape EV characteristics and decipher the microRNAs with modified expression for further in-vitro studies.
Is alpha-synuclein (α-synuclein) a foe for brain recovery after ischemic stroke?

Tizibt Ashine Bogale1, Domenico Mercurio4, Alessia Valente4, Serena Seminara4, Gaia Faustini1, Cristina Gussago1, Vanessa Porrini1, Marina Benarese1, Mariana Mota1, Sina Rhein5 Stefania Mitola2,3, Markus Schwaninger5,6, Stefano Fumagalli4, Arianna Bellucci1,3 and Marina Pizzi1

1Division of Pharmacology, Department of Molecular and Translational Medicine, University of Brescia, Viale Europa 11, 25123, Brescia, Italy; 2Biotechnology Division, Department of Molecular and Translational Medicine, University of Brescia, Viale Europa 11, 25123, Brescia, Italy; 3Laboratory for Preventive and Personalized Medicine, Department of Molecular and Translational Medicine, University of Brescia, Viale Europa 11, 25123, Brescia, Italy; 4Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Department of Neuroscience, Milan, Italy; 5Institute for Experimental and Clinical Pharmacology and Toxicology, Center of Brain, Behavior and Metabolism, University of Lübeck, Lübeck, Germany; 6DZHK (German Research Centre for Cardiovascular Research), Partner Site Hamburg/Lübeck/Kiel, Lübeck, Germany

Introduction and objectives: Recent evidence supports that α-synuclein, a protein widely implicated in the pathogenesis of Parkinson’s disease, is increased, and mediates brain damage following transient ischemia in spontaneously hypertensive rats (Kim et al., 2016). However, how α-synuclein contributes to brain damage following stroke remains unknown. Previous evidence supports a role for α-synuclein in modulating brain endothelial cells (Bogale et al., 2021). We thus investigated whether α-synuclein may impact on brain recovery post-ischemia by affecting vascular responses.

Results: We found a significant upregulation in total and Ser129 P-α-synuclein levels 7 days, but not 1 day after permanent middle cerebral artery occlusion (pMCAO) in wild type C57BL/6J mice. This was in line with a time-dependent increase of both total and Ser129 P-α-synuclein levels in neuronally-differentiated SK-N-SH cells following oxygen-glucose deprivation. Immunohistochemical analysis in the ischemic mouse brain revealed that α-synuclein mainly localized in neurons and macrophages, but not in astrocytes. When wild type mice (wt) were exposed to transient MCAO (tMCAO), no increase of α-synuclein was evident either at 2 or 7 days. To investigate the role of endogenous α-synuclein in brain vulnerability to ischemia, α-synuclein null mice were exposed to tMCAO. We found that 2 days after tMCAO, the null mice showed decreased expression of ICAM1, HIF1α, VEGFs, VEGFRs, MMP9, while at 7 days post-tMCAO, they exhibited more elevated expression of VEGFR2 and MMP9, and increased levels of tight-junction proteins and number of angiogenic vessels compared to wt animals. The α-synuclein null mice also exhibited a better survival compared to wt mice.

Conclusions: Our results indicate that induction of α-synuclein and its post-translational modification after stroke may depend on the duration and type of ischemia injury and suggest that endogenous α-synuclein may negatively influence both the early vascular response as well as the functional recovery in later phases of stroke by modulating vascular repair and remodelling.
Targeting the endocannabinoid and melatonergic systems to provide neuroprotection against the neuroinflammatory damage

Mariarosaria Cammarota¹, Silvia Rivara², Federica Vacondio², Gilberto Spadoni³, Marco Mor², Francesca Boscia¹

Division of Pharmacology, Department of Neuroscience, Reproductive, and Odontostomatological Sciences, School of Medicine, Federico II University of Naples, Naples, Italy. ²Department of Food and Drug, University of Parma; ³Department of Biomolecular Sciences, University of Urbino

Introduction and objectives: Regulating endocannabinoid (EC) levels by inhibiting the eCB-hydrolysing enzyme FAAH is an attractive therapeutic perspective in several neuroinflammatory and neurodegenerative diseases. Melatonin is a hormone released by the pineal gland that is currently used as a dietary supplement for the short-term treatment of insomnia. Both endocannabinoids and melatonin exert physiological brain functions and are provided by immunomodulatory, antioxidant, and protective roles. By employing a rodent hippocampal explant model of inflammatory injury we reveal the effects of UCM1341, a dual-acting compound with FAAH inhibitory action and agonistic activity on melatonin receptors, against the neuroinflammatory damage. FAAH activity and endocannabinoids levels were assessed by radiometric assays, and HPLC coupled to tandem mass spectrometry. FAAH distribution, and the contribution of UCM1341 to the inflammatory response were investigated by biochemical and confocal analyses.

Results: Our neuroprotection studies showed that the damage occurring in the CA1 region of hippocampal slices exposed to NMDA insult was not greatly affected by UCM1341. Conversely, the bivalent ligand attenuated demyelination and exerted a greater dose-dependent neuroprotection against the LPS+IFN-γ-induced neuroinflammatory damage if compared to the reference compounds melatonin or URB597. Confocal analysis revealed the early upregulation of FAAH protein in CA1 neurons and astrocytes after LPS+IFN-γ exposure. At later time points, FAAH persist in astrocytes, and increased in microglia surrounding damaged neurons. UCM1341 inhibited FAAH activity and augmented the levels of AEA and OEA. Moreover, it modulated the inflammatory response by contributing to microglia/macrophage polarization. The neuroprotective effects of UCM1341 were significantly prevented by the PPARα and melatonin receptor antagonists.

Conclusions: Our findings suggest that enhancing the EC and melatoninergic tone with UCM1341 may represent a novel strategy to provide neuroprotection and modulate the microglia/macrophages response after a neuroinflammatory demyelinating insult.

Bibliography:


Somatosensory processing deficits and altered connectivity in Cntnap2 -/- and Shank3b -/- mouse models of autism spectrum disorder.

Luigi Balasco1, Marco Pagani2, Luca Pangrazzi1, Gabriele Chelini1, Francesca Viscido1, Alessandra GC Chama1, Evgenia Shlosman1, Lorenzo Mattioni3, Alberto Galbusera2, Giuliano Iurilli4, Giovanni Provenzano3, Alessandro Gozzi2, Yuri Bozzi1

1 Center for Mind/Brain Sciences - CIMEC, University of Trento, 2 Functional Neuroimaging Laboratory, Center for Neuroscience and Cognitive Systems, Istituto Italiano di Tecnologia 3 Department of Cellular, Computational, and Integrative Biology - CIBIO, University of Trento 4 Systems Neurobiology Laboratory, Center for Neuroscience and Cognitive Systems, Istituto Italiano di Tecnologia.

Introduction and objectives: Abnormal tactile response is considered an integral feature of Autism Spectrum Disorders (ASDs - Robertson and Baron-Cohen, 2017; American Psychiatric Association 2013), and hypo-responsiveness to tactile stimuli is often associated with the severity of ASDs core symptoms (Foss-Feig et al. 2012). Mutations in the human CNTNAP2 and SHANK3 genes result in cortical dysplasia-focal epilepsy syndrome (CDFE - Strauss et al. 2006) and Phelan-McDermid syndrome (PMS – Phelan and McDermid 2012) respectively, two syndromic forms of autism. Likewise, Cntnap2-/- and Shank3b-/- mice show deficits relevant to core symptoms of human ASDs (Peñagarikano et al. 2011; Peça et al. 2011). Sensory abnormalities have been described in mice lacking ASD-associated genes (Balasco et al., 2019). However, the neural underpinnings of these somatosensory abnormalities are still poorly understood. Here we investigated, in Cntnap2-/- and Shank3b-/- mice, the neural substrates of whisker-mediated responses, a key component of rodents’ interaction with the surrounding environment.

Results: When compared to their controls, both Cntnap2-/- and Shank3b-/- mice displayed impaired whisker-dependent discrimination in the textured novel object recognition test (tNORT). Additionally, Shank3b-/- but not Cntnap2-/- mice showed a marked behavioural hypo-responsiveness to repetitive whisker stimulation in the whisker nuisance (WN) test. Notably, while Cntnap2-/- mice displayed increased c-fos mRNA induction within primary somatosensory cortex (S1) following whisker stimulation, Shank3b-/- mice showed a significantly reduced activation of S1. Moreover, when tested in a resting-state fMRI paradigm, Cntnap2-/- mice showed focal hyper-connectivity within the S1, while reduced S1-hippocampal connectivity was found in Shank3b-/- mice.

Conclusion: Together, these findings suggest that impaired neuronal activation and dysfunctional connectivity within S1 might underlie hypo-reactivity to whisker-dependent cues in Cntnap2-/- and Shank3b-/- mice, highlighting a potentially generalizable form of dysfunctional somatosensory processing in ASD.
Alterations in extracellular spaces thickness and dopaminergic activity in CKD mice Models

Antonio de Donato\textsuperscript{1,2}, Davide Viggiano\textsuperscript{1,2,3}

\textsuperscript{1} Dipartimento di Salute Mentale, Fisica e Medicina Preventiva, Università degli Studi della Campania “Luigi Vanvitelli”, Napoli; \textsuperscript{2} BIOGEM, Ariano Irpino; \textsuperscript{3} Dipartimento di Scienze Mediche Traslazionali, Università degli Studi della Campania “Luigi Vanvitelli”, Napoli

Introduction and objectives: Among all brain cells there is a continuous reticular compartment called extracellular space (ECS), characterized by extracellular fluid (Soria et al., 2020), a macromolecular network surrounding neurons and glia, defined extracellular matrix (Lam et al., 2019). ECS is responsible for the diffusion of soluble molecules in the brain, for homeostasis and metabolite clearance and serves as a channel for extrasynaptic signaling by volume transmission (Nicholson and Hrabetova, 2017). An alteration in ECS can negatively affect the processes associated with synaptic plasticity, learning and memory (Bosiacki et al., 2019) in which dopamine plays a pivotal role (Speranza et al., 2021). Reduced clearance of solutes and toxins in ECS is associated with various neurodegenerative diseases such as Alzheimer’s disease (Sun et al., 2021) and cognitive decline found in Diabetes (Kim et al., 2018). A high risk of developing dementia and mild cognitive impairment (MCI) has been found in patients with chronic kidney disease (CKD), a systemic disorder defined by a reduced kidney filtration capacity accompanied by changes in blood composition (with toxins accumulation) that can alter the neurotransmission in the central nervous system (CNS) (Viggiano et al., 2020). This study is focused on the analysis of ECS in the prefrontal cortex (PFC) and on dopaminergic activity through DARPP32 phosphoprotein target in the striatum of CKD animal models (mice 5/6 nephrectomy).

Results: Brains of intact mice and CKD mice (n=8 per group) were fixed. Cryosections were stained for DARPP32 and the ECS surrounding cortical interneurons evidenced using Wisteria Floribunda agglutinin. The ECS in PFC was significantly reduced and DARPP32 was significantly increased in the striatum of CKD animals.

Conclusions Our results demonstrate that the alteration of renal function in CKD reduces the ECS thickness. The alteration of ECS in CKD could be closely correlated with the alteration of dopaminergic neurotransmission in the striatum.
Mind the gap: a role for ER-mitochondria interaction, in Alzheimer’s disease astrocytes cellular dysfunction.

Giulia Dematteis¹, Laura Tapella¹, Marianna Moro¹, Beatrice Pistolato¹, Elisa Tonelli¹, Ambra Grolla¹, Riccardo Miggiano¹, Armando A Genazzani¹, Dmitry Lim¹

¹Department of Pharmaceutical Sciences, Università del Piemonte Orientale “Amedeo Avogadro”, Via Bovio 6, 28100, Novara, Italy;

Introduction and objectives: Calcium signalling deregulation, bioenergetic deficit and disproteostasis represent main features of early Alzheimer disease (AD) pathogenesis (Verkhratsky et al, 2016). Recently, we reported that this dysfunctions are present in immortalized hippocampal astrocytes from 3xTg-AD mice (3Tg-iAstro) in association with increased interaction between the endoplasmic reticulum (ER) and mitochondria (MIT) (Dematteis et al, 2020). It has been proposed that alteration of ER-MIT interaction, specifically the impairment of calcium transfer into mitochondria, plays a crucial role in pathogenesis of neurodegenerative disease, including AD (Lim, 2021). We hypothesized that the alteration of ER-MIT interaction, impairing ER-MIT calcium transfer, may be causative for AD related cellular dysfunction.

Results: To test this hypothesis we used a series of ER-MIT linkers (EML) that fix the distance between the organelles at 10 or 20 nm (EML-10nm; EML-20nm). We found that the overexpression of EML-10nm inhibits ER-MIT calcium transfer and faithfully replicates bioenergetic deficit and reduction of protein synthesis found in 3Tg-iAstro (Tapella et al, 2022). Instead, strikingly, EML-20nm, the linker which sets the optimal distance for ER-MIT calcium transfer, augmented ER-MIT calcium transfer and rescued the AD specific cellular pathology. These findings have been confirmed in a functional test, in which a tri-cell model of angiogenesis, composed of astrocytes, grown together with endothelial cells and pericytes (E/P), has been used. In detail, 3Tg-iAstro but not WT-iAstro severely impaired tubulogenesis when plated together with E/P in matrigel (Tapella et al, 2022); WT-iAstro expressing EML-10nm impair the formation of tubules, while the expression of EML-20nm in 3Tg-iAstro rescues the tubulogenesis when plated together with E/P.

Conclusions: In summary, our results suggest a causal role for the increased MIT-ER interaction in AD related astroglial dysfunction, indicating that normalizing calcium transfer between ER-MIT could represent a valuable strategy for AD treatment.
In vivo neurotransmitter deficits in genetic frontotemporal dementia. A GENFI study


1. Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy; 2. Stroke Unit, Azienda Socio Sanitaria Territoriale Spedali Civili Brescia, Brescia, Italy; 3. Institute of Neuroscience and Medicine, Brain & Behaviour (INM-7), Research CentreJülich, Jülich, Germany; 4. Institute of Systems Neuroscience, Medical Faculty, Heinrich Heine University Düsseldorf, Düsseldorf, Germany; 5. Centre for Neurodegenerative Disorders, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy; 6. Neurophysiology Unit, Azienda Socio Sanitaria Territoriale Spedali Civili Brescia, Brescia, Italy; 7. Neuroradiology Unit, University of Brescia, Brescia, Italy; 8. Laboratory Unit, Azienda Socio Sanitaria Territoriale Spedali Civili Brescia, Brescia, Italy; 9. Department of Neurodegenerative Disease, Dementia Research Centre, UCL Institute of Neurology, Queen Square, London; 10. Department of Neurology, Erasmus Medical Centre, Rotterdam, Netherlands; 11. Alzheimer's disease and Other Cognitive Disorders Unit, Neurology Service, Hospital Clinic, University of Barcelona, Barcelona, Spain; 12. Cognitive Disorders Unit, Department of Neurology, Donostia University Hospital, San Sebastian, Gipuzkoa, Spain; 13. Clinique Interdisciplinaire de Mémoire, Département des Sciences Neurologiques, CHU de Québec, and Faculté de Médecine, Université Laval, QC, Canada; 14. Center for Alzheimer Research, Division of Neuroimaging, Department of Neurobiology, Care Sciences and Society, Bioclinicum, Karolinska Institutet, Solna, Sweden; 15. Department of Neurodegenerative Diseases, Hertie-Institute for Clinical Brain Research and Center of Neurology, University of Tübingen, Tübingen, Germany; 16. Fondazione Ca' Granda, IRCCS Ospedale Policlinico, Milan, Italy; 17. University of Milan, Centro Dino Ferrari, Milan, Italy; 18. Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK; 19. Sunnybrook Health Sciences Centre, Sunnybrook Research Institute, University of Toronto, Toronto, Canada; 20. Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Toronto, Canada; 21. Department of Clinical Neurological Sciences, University of Western Ontario, London, Ontario Canada; 22. Laboratory for Cognitive Neurology, Department of Neurosciences, KU Leuven, Leuven, Belgium; 23. Neurology Service, University Hospitals Leuven, Leuven, Belgium; 24. Leuven Brain Institute, KU Leuven, Leuven, Belgium; 25. Laboratory of Neurosciences, Institute of Molecular Medicine, Faculty of Medicine, University of Lisbon, Lisbon, Portugal; 26. Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy; 27. Nueld Department of Clinical Neurosciences, Medical Sciences Division, University of Oxford, Oxford, UK; 28. University Hospital of Coimbra (HUC), Neurology Service, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; 29. Division of Neuroscience and Experimental Psychology, Wolfson Molecular Imaging Centre, University of Manchester, Manchester, UK; 30. Sorbonne Université, Paris Brain Institute – Institut du Cerveau – ICM, Inserm U1127, CNRS UMR 7225, AP-HP - Hôpital Pitié-Salpêtrière, Paris, France; 31. Centre de référence des démences rares ou précoces, IM2A, Département de Neurologie, AP-HP - Hôpital Pitié-Salpêtrière, Paris, France; 32. Département de Neurologie, AP-HP – Hôpital Pitié-Salpêtrière, Paris, France; 33. Reference Network for Rare Neurological Diseases (ERN-RND); 34. Department of Psychiatry, McGill University Health Centre, McGill University, Montreal, Quebec, Canada; 35. Neurologische Klinik, Ludwig-Maximilians-Universität München, Munich, Germany; 36. Department of Neurology, University of Ulm, Ulm, Germany; 37. Department of Neurofarba, University of Florence, Italy; 38. IRCCS Fondazione Don Carlo Gnocchi, Florence, Italy;
**Introduction and objectives.** Frontotemporal dementia (FTD) encompasses a spectrum of neurodegenerative diseases characterized by progressive behavioral and personality changes, executive dysfunctions and language impairment (Bang et al, 2015). In around 25-35% of cases FTD is genetically determined, with microtubule-associated protein tau (MAPT) and progranulin (GRN) mutations, and chromosome 9 open-reading-frame 72 (C9orf72) expansion as major pathogenetic determinants (Borroni et al, 2013).

At present, there is a lack of studies exploring in vivo neurotransmitter deficits in genetic FTD; however, detailed knowledge of neurotransmitters impairment holds the potential to investigate pathogenesis and develop new tailored therapeutic approaches.

In the present study we applied JuSpace toolbox, which allowed for cross-modal correlation of Magnetic Resonance Imaging (MRI)-based measures with nuclear imaging derived estimates covering various neurotransmitter systems including dopaminergic, serotonergic, noradrenergic, GABAergic, glutamatergic and opioid neurotransmission (Dukart et al, 2021). In GENFI (Genetic Frontotemporal Dementia Initiative) cohort, we tested if the spatial patterns of grey matter volume (GMV) alterations in presymptomatic and symptomatic mutation carriers, compared to healthy controls (HC) were correlated with specific neurotransmitter systems.

**Results.** We included 276 HC, 309 presymptomatic (117 C9ORF72, 136 GRN and 56 MAPT) and 98 symptomatic mutation carriers (33 C9ORF72, 39 GRN and 26 MAPT).

As compared to HC, presymptomatic and symptomatic mutation carriers showed patterns of grey matter atrophy in line with literature data (Cash et al, 2018). We did not find any significant association between voxel-based brain changes in presymptomatic patients and the spatial distribution of the different neurotransmitter systems considered. On the other hand, as compared to HC, brain changes in symptomatic mutation carriers were significantly associated with spatial distribution of serotonin, dopamine, GABAergic, glutamatergic and opioid pathways ($p<0.05$, False Discovery Rate corrected for multiple comparisons).

**Conclusions.** This study suggests that JuSpace is a helpful tool to indirectly assess neurotransmitter deficits and explore pathophysiology in neurodegenerative dementias.
Insights into the neurodegenerative mechanisms associated with KIF5A mutations

Marta Cozzi1, Barbara Tedesco1,2, Riccardo Cristofani1, Veronica Ferrari1, Elena Casarotto1, Marta Chierichetti1, Francesco Mina1, Paola Pramaggiore1, Margherita Piccolella1, Mariarita Galbiati1, Paola Rusmini1, Valeria Crippa1, Cinzia Gellera2, Stefania Magri2, Serena Santangelo3,4, Antonia Ratti3,4, Francesco Mina1, Paola Rusmini1

Introduction and objectives: KIF5A is a neuron-specific kinesin driving anterograde axonal transport. It comprises an N-terminal motor domain for ATP-dependent microtubule binding, a coiled-coil stalk for conformational changes and dimerization, and a C-terminal tail domain for cargo binding and autoinhibition (Hirokawa et al., 2009). Mutations targeting the three KIF5A domains give rise to distinct neurodegenerative diseases (Sleigh et al., 2019), but the processes underlying such phenotypic heterogeneity are not yet known. Our aim is to investigate the pathogenetic mechanisms behind KIF5A-related neurodegeneration by functionally characterizing four disease-associated KIF5A mutations (R17Q, R280C, R864X, N999Vfs*39), which target the different domains of the protein.

Results: Altered protein turnover was evidenced for R17Q and N999Vfs*39 KIF5A upon overexpression, with the two mutants displaying shorter half-life compared to the wild-type (WT) protein. At the same time, R864X and N999Vfs*39 KIF5A showed abnormal intracellular distribution by preferentially localizing within neurites instead of being diffused in the whole cytoplasm like WT KIF5A. Such aberrant distribution pattern is consistent with impaired R864X and N999Vfs*39 KIF5A autoinhibition, respectively depending on loss or alteration of KIF5A tail domain. More in detail, while the R864X mutant was diffused within neurites, N999V*fs39 KIF5A formed puncta colocalizing with the ubiquitin-binding protein p62. Proteasomal blockage induced significant R17Q and N999Vfs*39 KIF5A accumulation within detergent-insoluble inclusions, indicating that the two mutants are mainly degraded by the ubiquitin-proteasome system and that they may form harmful aggregates when proteostasis is impaired. On the other hand, no involvement of the autophagic pathway was found in mutant KIF5A degradation. Finally, the abnormal distribution pattern characterizing R864X and N999Vfs*39 KIF5A was paralleled by limited colocalization with mitochondria, whose axonal transport largely relies on KIF5A, and by WT KIF5A sequestration within neurites.

Conclusions: Together, our preliminary results suggest that both unique and shared pathogenetic mechanisms underpin mutant KIF5A-dependent neurodegeneration.

Bibliography:
Synaptic targets of KLVFF: the impact on the neurotransmitters release.
Hanna Trebesova¹, Guendalina Olivero¹, Mario Marchi¹, Massimo Grilli¹
¹Department of Pharmacy, Section of Pharmacology and Toxicology, University of Genoa

Introduction and objectives: The aggregation of beta-amyloid (Aβ) is one of the critical factors in the pathogenesis of Alzheimer’s disease, therefore one therapeutic strategy can be the inhibition of this mechanism. Accordingly, a lot of preclinical studies investigated promising Aβ aggregation inhibitors including metal chelators, nanostructures, organic molecules, peptides, and antibodies. KLVFF is a small peptide corresponding to the aminoacidic sequence 16-20 of Aβ that reduced fibrillation in a dose-dependent manner (Mitra and Sarkar 2020). Its pharmacological characterization is fundamental for elucidating the potential therapeutic role. Accordingly, we investigated the modulatory role of KLVFF on native cholinergic receptors expressed on rat synaptosomes. Moreover, we assessed the direct toxicity of KLVFF on synaptic terminals through calcein-AM and annexin-V labelling followed by flow cytometric analysis.

Results: In the nucleus accumbens the nicotinic receptors on dopaminergic nerve terminals are inhibited by KLVFF, that closely resembles the full-length Aβ1-40. Interestingly, KLVFF entrapped in synaptosomes is ineffective, suggesting that the external binding to the receptor is required for its activity. Furthermore, the cholinergic agents desformylflustrabromine and galantamine counteracted the KLVFF effect. Remarkably, muscarinic receptors on dopaminergic terminals and nicotinic receptors regulating noradrenaline release in the hippocampus are completely insensitive to KLVFF. Based on our findings, KLVFF mimics Aβ1-40 as a negative modulator of specific nicotinic receptor subtypes affecting dopamine transmission in the rat’s brain (Olivero et al. 2014). In addition, the in/out presence of KLVFF does not modify the viability of synaptosomes

Conclusions: The anti-aggregative peptide KLVFF modulates specific nicotinic receptor subtypes without evident toxic effects on the synaptic terminals. Further investigation should be carried out to evaluate the impact of this activity on Alzheimer’s disease patients.
Insights into Frontotemporal Dementia pathogenesis: the role of anti-GluA3 antibodies

Maria Italia, Diego Scheggia, Elisa Zianni, Michela Salvadé, Monica Di Luca, Alessandro Padovani, Barbara Borroni, Fabrizio Gardoni

Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano

Introduction and objectives: Frontotemporal dementia (FTD) is a common type of presenile dementia that presents as a clinically and neuropathologically heterogeneous disorder. Recently, autoantibodies directed against the GluA3 subunit of AMPA-type glutamate receptors (AMPARs) have been identified in 20% to 25% of FTD patients (Borroni et al., 2017). Data from patients and in vitro/ex vivo studies indicate that anti-GluA3 IgG negatively affect glutamatergic neurotransmission (Borroni et al., 2017; Palese et al., 2020; Scheggia et al., 2021).

We studied whether and how the chronic presence of anti-GluA3 IgG triggers a neurodegenerative process in mice and the association between this process and the appearance of FTD-related neuropathological and behavioral signature. What is more, we investigated a possible rescue strategy to counteract the detrimental effects mediated by anti-GluA3 IgG.

Results: We developed a chronic mouse model of autoimmunity in FTD by infusing mice with anti-GluA3 IgG isolated from FTD patients for one month through an intracerebroventricular cannula. The model was used to perform morphological and biochemical analyses and behavioral tasks. As a rescue strategy, we treated mice with a well-validated AMPAR positive allosteric modulator (PAM) (Bretin et al., 2017; Mendez-David et al., 2017).

Data showed that chronic anti-GluA3 IgG administration led to the appearance of FTD-related neuropathological markers and to dendritic spine loss in mice prefrontal cortex. In addition, we identified alterations in sociability and cognition that partially reflect those deficits proper of FTD GluA3+ patients. Some of these alterations were rescued by PAM administration.

Conclusions: Our model allowed to identify the specific contribution of anti-GluA3 autoantibodies to FTD neuropathology. In addition, the model was instrumental to develop a putative therapeutic strategy for GluA3+ patients.

Bibliography:


Long-term effect after intravenous self-administration (IVSA) of the synthetic cannabinoid receptor agonist 5F-MDMB-PICA in adolescent mice

Francesca Caria
Department of Biomedical Sciences, University of Cagliari, 09042 Cagliari, Italy.

Introduction and objectives: Synthetic cannabinoids (SC) are the largest group of new psychoactive substances monitored worldwide (EMCDDA; DEA; NIDA, NDARC 2019-22). 5F-MDMB-PICA is a SC recently detected and classified as a potent agonist of CB1 and CB2 receptors able to activate the mesolimbic dopamine transmission in adolescent (0.01 mg/kg ip) but not in adult mice (Musa et al, 2020). In this study, we aimed: i) to investigate the reinforcing properties and abuse potential of the SC 5F-MDMB-PICA in adolescent mice; ii) to characterize the behavioral effects induced in adult mice by 5F-MDMB-PICA IVSA during adolescence by means of the resident intruder paradigm and the taste reactivity test.

Results: The response-study to varying doses showed an inverted U-shaped trend. Adolescent mice acquired operant behavior for 5F-MDMB-PICA at the dose of 2.5 (μg/kg/25 ul), with significant difference between the number of active and inactive lever pressing performed. This dose was then used under different ratio of responding (FR1-3, PR), thus showing that the behavior was directed to obtain the SC. Behavioral differences were identified in relation to the 5F-MDMB-PICA intake during adolescence. In particular, we showed that a total intake of 5F-MDMB-PICA higher than 15 ug/kg (over 15 sessions) during adolescence induced a propensity for aggressive behavior and a reduced social interaction at adulthood. No differences were observed in the behavioral response to natural rewarding taste stimuli (chocolate).

Conclusions: This study provides the first evidence that 5F-MDMB-PICA IVSA is acquired and sustained by adolescent mice within a narrow window of doses. The dose sufficient to sustain operant behavior was lower than that of the prototypical SC JWH-018, confirming the abuse liability of this newer SC. Moreover, 5F-MDMB-PICA IVSA during adolescence induced behavioral changes at adulthood. Our study increases the knowledge of the consequences related to the use of SC by young people.
Cognitive decline induced by intracerebral infusion of human-alpha synuclein oligomers is associated with altered neuronal firing in cingulate cortical neurons. M Francesca Palmas¹, Michela Etzi¹, Claudia Sagcheddu¹, Michele Santoni¹, Chiara Camoglio¹, Giuliana Fusco², Alfonso de Simone³, Luca Picci⁴, Paola Fadda¹, Marco Pistis¹, Nicola Simola¹, Ezio Carboni¹, Augusta Pisanu⁵, Anna R Carta¹

¹Department of Biomedical Sciences, University of Cagliari, Italy; ²Centre for Misfolding Diseases, Department of Chemistry, University of Cambridge, Cambridge, UK; ³Department of Pharmacy, University of Naples “Federico II”, Italy; ⁴Department of Life and Environmental Sciences, University of Cagliari, Italy; ⁵National Research Council, Institute of Neuroscience, Cagliari, Italy

Introduction and objectives: Cognitive dysfunction represent one of the most disabling non-motor symptoms of Parkinson's disease (PD), though, so far, its pathological correlates still remain elusive, mainly for the lack of a valid preclinical neuropathological model that reproduces both motor and non-motor aspects of the disease (Chaudhuri & Odin, 2010). Several clinical studies reported the presence of a number of inflammatory markers in parkinsonian patients' brains, pointing at the neuroinflammation as a contributing factor in the development of cognitive decline (Kouli et al., 2020). Here, we show that the bilateral intracerebral infusion of pre-formed human alpha synuclein oligomers (H-αSynOs) within the substantia nigra pars compacta (SNpc) offers a valid model for studying this aspect of the pathology.

Results: Specifically, three months post-surgery, H-αSynOs-infused rats developed a severe cognitive decline both in the short-term spatial reference memory and in the short-term recognition memory. This cognitive impairment was associated with an altered inflammatory response in the anterior cingulate cortex (aCxg) and in discrete subfields of the dorsal hippocampus, as revealed by the increase in GFAP immunoreactivity and the acquisition of a proinflammatory phenotype by microglial cells. Indeed, we found increased number of microglial cells expressing large amount of the proinflammatory cytokine TNF-α as compared to vehicle-infused rats, supporting a role of neuroinflammation in this symptomatic aspect. In addition, we observed alterations in electrophysiological parameters in the aCgx, as demonstrated by the reduced firing activity of putative pyramidal neurons in vivo.

Conclusions: All together, we present a neuropathological rat model of PD that recapitulates cognitive symptoms of PD. The intranigral infusion of toxic oligomeric species of alpha-synuclein induces a neuroinflammatory environment in distant cognitive relevant regions, resulting in an altered neuronal activity that may account for the cognitive deficits.

Bibliography:

SARS-COV2’s tropism in neurons and glia impairs neuronal networks activity: an in vitro study on rat cortical cells
Matteo Manzati1, Diletta Pozzi1, Pamela Martinez-Orellana2, Valentina Perrera1, Alessandro Marcello2, Michele Giugliano1
1 International School for Advanced Studies (SISSA); 2 International Centre for Genetic Engineering and Biotechnology (ICGEB)

Introduction and objectives: Brain autopsies of deceased patients showed co-localization of SARS-CoV-2 and cortical lesions (Song et al., 2020). A recent biochemical analysis of the plasma of severe and moderate COVID-19 cases demonstrated an increase in biomarkers of CNS lesions (Kanberg et al., 2020). We examined the effects of SARS-Cov2 infection on neurons in an ex vivo model of rat brain cortical networks.

Results: For the first time, we report that rat neurons and glial cells are susceptible to SARS-Cov2 infection, even if lacking hACE2 receptors. We observed nucleocapsid-positive cells as early as 3h post-infection. At 16 and 24h post-infection, glial cells showed a significantly increased rate of infection, compared to neurons. We analyzed the electrophysiological phenotype of infected neuronal networks by microelectrode arrays (MEAs) recordings and found altered electrical signaling and subsequent irreversible signal loss. At 24h post-infection both spontaneous and electrically induced generation of neuronal impulses and coordinated network activity was reduced to zero. At 3h post-infection, extracellular electrical stimuli still induced low-latency action potentials, suggesting that antidromic (axonal) excitability was still preserved. However, the complete disappearance of “reverberating” electrophysiological events at larger latencies, strongly expressed before infection, suggests that a loss of synaptic function is occurring.

Conclusions: We hypothesized that such a scenario results from inflammatory events including microglia activation. A similar but weaker downregulation of spontaneous electrophysiological events was observed in cultures exposed to UV-inactivated SARS-Cov2 virus, in which replication was impaired but the spike protein and its membrane binding were preserved. This result suggests that membrane binding of SARS-Cov2 is sufficient to trigger inflammatory events. Cytokines analysis revealed a key role of TGF-β, TNF-α, IL-1α, RANTES and MIP-1β. Future studies aimed at preventing the neurological symptoms of long–covid syndrome could target the inhibition of these cytokines and benefit from our initial analysis of the impairments on the electrophysiological activity.

Bibliography:
Mitochondrial alterations in subjects with idiopathic REM sleep disorders as a predictive biomarker for conversion to Parkinson’s disease

Gerardo Ongari1,2, Silvia Cerri1, Micol Avenali3,4, Michele Terzaghi5, Fabio Blandini1,3

1 Unit of Cellular and Molecular Neurobiology, IRCCS Mondino Foundation, Pavia; 2 Department of Medicine and Surgery, University of Insubria, Varese; 3 Department of Brain and Behavioural Sciences, University of Pavia, Pavia; 4 Neurorehabilitation Unit, IRCCS Mondino Foundation, Pavia; 5 Unit of Sleep Medicine and Epilepsy, IRCCS Mondino Foundation, Pavia

Introduction and objectives: Idiopathic REM sleep disorders (iRBD) are the most important prodromal marker of Parkinson’s disease (PD). Nevertheless, only few studies so far investigated the mechanisms potentially involved in the development of PD in iRBD subjects. Since the presence of mitochondrial dysfunctions have been linked to sleep disturbances in PD (Smith et al., 2018; Milanese et al., 2019), in this project we explored the potential mitochondrial alterations in fibroblasts of subjects with iRBD, in order to identify a biochemical profile that can characterize this condition and that can allow to predict the future onset of PD.

Results: The project involved 23 subjects divided into three experimental groups: healthy subjects, subjects with iRBD and iRBD subjects subsequently converted to PD (iRBD-PD).

The evaluation of the mitochondrial metabolism in the fibroblasts of iRBD subjects by the XFe24 Seahorse Analyzer, revealed a reduction in maximal and spare respiration levels compared with healthy controls, though not statistically significant. Instead, a significant worsening of the bioenergetic profile in iRBD-PD subjects' cells was observed, as evidenced by the decrease of ATP production and of basal, maximal and spare respiration levels. The impairment of mitochondrial function in iRBD-PD patients is associated with a significant decrease in the expression levels of electron transport chain complexes III and V and the presence of mitochondrial fragmentation. iRBD subjects showed similar, but less severe, alterations.

Conclusions: These findings suggest that mitochondrial alterations (e.g., the reduced ability to respond to increased energy demand) observed in the fibroblasts of iRBD subjects may predispose to the worsening of the bioenergetic profile in iRBD subjects already converted to PD, thus indicating a potential mechanism underlying the progression of PD in iRBD patients.
Extracellular vesicles cooperate with PQC system for the clearance of TDP-43 species associated with ALS and FTD

Elena Casarotto1, Daisy Sproviero2, Eleonora Corridori1, Maria C. Gagliani3, Marta Cozzi1, Marta Chierichetti1, Riccardo Cristofani1, Veronica Ferrari1, Mariarita Galbiati1, Francesco Mina1, Margherita Piccolella1, Paola Rusmini1, Barbara Tedesco1,4, Stella Gagliardi2, Katia Cortese3, Cristina Cereda2, Angelo Poletti2, Valeria Crippa1

1 Dipartimento di Scienze Farmacologiche e Biomolecolari (DiSFeB), Department of Excellence 2018-2022, Università degli Studi di Milano, via Balzaretti 9, 20133 Milano (MI), Italy; 2 Genomic and post-Genomic Center, IRCCS – Mondino Foundation, via Mondino 2, 27100 Pavia (PV), Italy; 3 Department of Experimental Medicine (DIMES), Cellular Electron Microscopy Lab, University of Genova, via Antonio de Toni 14, 16132 Genova (GE), Italy; 4 Unit of Medical Genetics and Neurogenetics, Fondazione IRCCS – Istituto Neurologico “Carlo Besta”, via Celoria 11, 20133 Milano (MI), Italy

Introduction and objectives: ALS and FTD are two neurodegenerative diseases characterized by the presence within neurons of abnormal cytoplasmic inclusions containing the insoluble forms of the TAR DNA-binding protein of 43 KDa (TDP-43) and of its C-terminal fragments of 35 (TDP-35) and 25 KDa (TDP-25) (Neuman et al., 2006). These TDP inclusions are toxic and impair cell functionality (Berning et al., 2019). Cells can remove TDP species (TDPs) thanks to the protein quality control (PQC) system [i.e. chaperone proteins, ubiquitin-proteasome system (UPS) and autophagy]. Recently, TDPs have been also found into extracellular vesicles (EVs) suggesting that EVs may have a role in TDPs clearance (Iguchi et al., 2016). This observation led us to wonder if a possible interplay between PQC and EVs in TDPs disposal may exist. To answer this question, we pharmacologically blocked PQC in an immortalized neuronal cell line and analysed the effect on their released EVs in terms of number and protein cargo, with special attention to TDPs and some PQC components involved in TDPs disposal [i.e. the chaperone assisted selective autophagy (CASA) complex proteins (HSP70-CHIP-HSPB8-BAG3), MAP1LC3B and SQSTM1/p62] (Crippa et al., 2016).

We considered all EVs, both large and small vesicles (LVs and SVs).

Results: All TDPs are physiologically secreted in EVs, mainly as insoluble species. EVs also transported CASA proteins, MAP1LC3B and SQSTM1/p62. PQC blockage increased the number of secreted EVs and their TDPs content, particularly in LVs. Interestingly, this increase was paralleled by the enrichment of CASA complex proteins.

Conclusions: In conclusion, EVs could represent an important mechanism for the clearance of insoluble TDPs which is specifically boosted when PQC is impaired and the CASA complex members could have a role in TDPs targeting to EVs.

Bibliography:


Improved synapsins phosphorylation and alpha-synuclein nitration occur in concert in experimental models of Parkinson’s disease

Viviana Brembati¹, Gaia Faustini¹, Francesca Longhena¹, Giulia Abate¹, Daniela Uberti¹, Tiago Fleming Outeiro², Arianna Bellucci¹

1 Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy; 2 Department of Experimental Neurodegeneration, University Medical Center Göttingen, Göttingen, Germany

Introduction and objectives: Parkinson’s disease (PD) is characterized by the loss of nigrostriatal dopaminergic neurons and the deposition of α synuclein (α-syn) in Lewy’s bodies (LBs) (Spillantini et al. 1997). Our research team recently described that α-syn interacts and cooperates with synapsin III (syn III), a synaptic vesicle associated phosphoprotein, to regulate dopamine release (Zaltieri et al., 2015). Moreover, we showed that syn III is a key component of α-syn fibrils extracted from the post-mortem brain of PD patients (Longhena et al., 2018) and it is also crucially involved in the control of α-syn aggregation (Faustini et al., 2018; Faustini et al., 2022).

Since different post translational modifications (PTMs) have been found to affect both α-syn and syn III structure and function (Oueslati et al., 2010; Longhena et al., 2021), the aim of the study is to dissect the impact of post translational modifications (PTMs) on α-syn/syn III interplay in cellular and in vivo models of PD.

Results: Interestingly, we found that the overexpression of human EGFP-tagged syn III increased the levels of endogenous α-syn in SK-N-SH cells, further supporting that syn III promotes α-syn pathology. In addition, we observed that the adeno associated viral vector (AAV)-mediated overexpression of human α-syn in the nigrostriatal system of C57BL/6J mice increased levels of syn III in both the substantia nigra and striatum but led to a significant improvement of phosphorylated synapsins only in the latter brain area. A significant increase of tyrosine 125 and 136 nitrated α-syn was also observed in the mice injected with the AAV inducing human α-syn overexpression.

Conclusions: These findings support that overexpression of α-syn is linked with an increase in oxidative injury and with an enhanced phosphorylation of synapsins and suggest that α-syn and syn III PTMs could drive α-syn accumulation-related synaptic toxicity.
ORAL SESSION
Cognition and behavior
The acute effects of intrusive thinking on neurotransmission within anterior cingulate cortex in pathological and non-pathological worriers: A 1H magnetic resonance spectroscopy and ecological momentary assessment study

Martino Schettino¹,², Chiara. Parrillo³, Simone Gazzellini³, Federico. Giove⁴,², Antonio Napolitano³, Cristina Ottaviani¹,²

¹ Department of Psychology, Sapienza University of Rome, Rome, Italy; ² IRCCS Santa Lucia Foundation, Rome, Italy; ³ Bambino Gesù Children’s Hospital, Rome, Italy; ⁴ Centro di Studi e Ricerche Enrico Fermi, Rome, Italy

Introduction and objectives: Alterations in glutamatergic and GABAergic neurotransmission are posited to be implicated in the pathophysiology of stress-related disorders (e.g., Brambilla et al., 2003; Lener et al., 2017). However, in humans existing evidence is inconsistent (e.g., Moriguchi et al., 2019 for a meta-analysis). Indeed, comparisons between pathological and healthy individuals are primarily at rest and not during specific disease states, making it difficult to understand processes underpinning transdiagnostic psychiatric symptoms. The present study applied ¹H magnetic 3T-resonance spectroscopy in the anterior cingulate cortex to investigate the effects of an experimental induction of intrusive thinking on glutamate (Glx, glutamate/glutamine ratio) and GABA in pathological worriers and controls (n = 33; 15 males). The ecological validity of the results has been tested by ecological momentary assessment of intrusive thinking on the same participants.

Results: Results showed an increase in regional levels of GABA from pre to post induction in both pathological and healthy individuals. Moreover, an opposite pattern emerged for Glx where the results showed a statistically significant Time x Group interaction (for Glx/Water: F₁,₂₁ = 4781, p = .04, η² = .18; for Glx/Cre: F₁,₂₁ = 4867, p = .03, η² = .17) with a pre to post increase in controls (p < .05) and a pre to post decrease in worriers (p < .05). Resting levels of GABA and Glx predicted subjective responses to the induction, namely levels of intrusiveness and repetitiveness (all ps < .05). Pre to post changes in neurotransmission within ACC also predicted daily life levels of thoughts intrusiveness and repetitiveness (all ps < .05).

Conclusions: Enhanced levels of regional GABA may indicate increased effort to suppress intrusive thoughts in both groups. Moreover, changes in Glx levels indicates that intrusive thinking has the effect of making healthy individuals neurochemically like disordered individuals (e.g., Makovac et al., 2016). These preliminary findings suggest that glutamatergic dysfunctions may contribute to the maintenance of intrusive thinking in pathological worriers and may inform personally-tailored treatments in the framework of precision psychiatry.
Evidence of vestibular contributions to visuospatial attention in patients with vestibular disorders

Roberto Gamberi1, Adriana Salatino1,2, Sergio Lucisano3, Roberto Albera4, Emanuele Cirillo5, Selene Schintu5, Hilary Serra1, Anna Berti1, Raffaella Ricci1

1 Department of Psychology, University of Turin, Via Verdi 10, Torino, Italy; 2 Institute of Neuroscience (IoN), Université Catholique de Louvain Brussels, Brussels, Belgium; 3 AOU Città della salute e della Scienza of Torino Hospital, corso Bramante 88, 10100, Turin, Italy; 4 Department of Surgical Sciences, University of Turin, Corso Dogliotti 14, 10100, Turin, Italy.

Introduction and objectives: Although some studies have shown that the vestibular system is engaged in visuospatial attention [Bottini et al., 2001; Dieterich et al., 2018], its specific contribution to its endogenous and exogenous components is largely unknown. To address this issue, a group of patients with peripheral vestibular disorders (VD) and a control group of age-matched healthy participants performed the Endogenous and Exogenous cue-to-target Posner Task. Participants had to detect, as quickly and accurately as possible, the presence of peripheral visual targets (either on the right or on the left of the screen) that could be preceded by valid, invalid or neutral cues. Central predictive cues (i.e., 80% of valid trials) endogenously orient attention, while peripheral non-predictive cues (i.e., 50% of valid trials) exogenously attract attention.

Results: In the Exogenous task, both patients and controls showed a validity effect without differences between groups. Both groups were faster in detecting targets in valid compared to neutral (p<.0001) and invalid (p<.0001) trials, and in neutral compared to invalid (p<.0001) trials. Conversely, in the Endogenous task a significant interaction showed that only controls were faster in valid trials compared to neutral (p<.011) and invalid (p<.0001) trials, and in neutral compared to invalid (p<.0001) trials, whereas patients did not show any effect of cue validity.

Conclusions: A selective disruption of voluntary orienting of visuospatial attention was found in VD-patients, while automatic attention was spared. Interestingly, these findings are consistent with recent data showing weak voluntary orienting of visuospatial attention when the vestibular system is unweighted in zero gravity [Salatino et al., 2021] and with neuroanatomical evidence in VD-patients displaying altered structures of the endogenous attention network [Dieterich et al., 2018]. Although our findings warrant further investigation, they shed new light on the influence of vestibular function on attentional processes.

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Dissecting the neural bases behind observational learning of complex social behaviors
Filippo La Greca, Elisa Zianni, Jennifer Stanic, Nicolò Carrano, Maria G. Coccia, Fabrizio Gardoni, Monica Di Luca, Diego Scheggia
Laboratorio di Farmacologia della Neurodegenerazione, Dipartimento di Scienze Farmacologiche e Biomolecolari, Sperimentali e Cliniche (DISFeB), Università degli Studi di Milano (Unimi)

Introduction and objectives: Humans constantly observe and learn from others, this phenomenon called observational learning (Bandura, 1977). Through observation, humans model others’ movements, emotions and even complex social behaviors (Ramsey et al., 2021). Rodent studies have helped defining the neural circuitry underlying observational learning of spatial and emotional skills (Olsson et al., 2020). However, whether rodents can learn refined social behaviors through observation and what are the areas involved is still unknown. The hippocampus is crucial for learning processes and has a key role in the representation of others’ location-action in the space (Danjo et al., 2018; Omer et al., 2018). Said that, hippocampal changes or deficits might compromise observational learning ability in social contexts (Schafer et al., 2018). The major goal of the current PhD project is dissecting the neural bases underlying observational learning of complex social behaviors. We studied observational learning in mice focusing on the role of the dorsal hippocampus (ie, dorsal CA1). We also analyzed observational learning in a model of Alzheimer’s Dementia (AD) characterized by hippocampal dysfunction (ie, APP/PS1 mice).

Results: We used an operant social decision-making task where mice observe their conspecifics and learn to make prosocial or selfish choices (ie, sharing or withholding food). We investigated the role of dorsal CA1 by inhibitory chemogenetics (Roth, 2016). Then, we tested APP/PS1 mice in our task. Our results show that mice, after observation, display better learning compared to mice without observation. Chemogenetic silencing of dorsal CA1 during the observational phase reverses this advantage producing deficits at start. Similarly, APP/PS1 mice exhibit impairments in observational learning ability.

Conclusions: Our study starts addressing the important role of dorsal CA1 in observational learning of complex social behaviors. Besides, our behavioral results with a model of AD highlight how hippocampal dysfunction might compromise observational learning potentially disrupting social life.

Bibliography:
ORAL SESSION
Development
Semaphorin-3A drives axonal growth cone elongation during neuronal development in human neural progenitors
Ferretti Gabriella¹, Romano Alessia², Sirabella Rossana¹, Serafini Sara¹ and Matrone Carmela¹
¹ Unit of Pharmacology, Department of Neuroscience, Faculty of Medicine, University of Naples Federico II, Naples, Italy; ² Ceinge, Faculty of Medicine, University of Naples Federico II, Naples, Italy

Introduction and objectives: Semaphorins are repellent guidance cues essential in guiding axons and connecting with one another and their targets during nervous system development (Limoni et al., 2020). Class 3 Sema (Sema-3) are the only produced as secreted proteins in mammals, exerting both autocrine and paracrine functions (Alto et al. 2017). Among the others, Sema-3A is one of the best characterized subtypes of this family, inducing growth cone elongation or collapse depending on the neuronal population in which it is expressed (Tessier-Lavigne et al., 1997). This suggests that changes in Sema 3A pathway might result in neurodevelopment defects. Indeed, increased Sema-3A expression levels have been detected in patients with autism or schizophrenia (Eastwood et al. 2003, Mah et al. 2006, Schafer et al. 2019) and polymorphisms in Sema-3A or in Sema-3A receptors, Neuropilin-1 (Npn-1) and Plexin-As (Plxn-A2), have been associated to neurodevelopmental disorders (van der Klaauw et al. 2019). We here questioned how increased Sema-3A influences the early stages of neuronal development using human neural progenitors.

Results: Sema-3A overexpression causes axon growth cone retraction and induces an aberrant proximal dendritic branching in NP. Both these events can be prevented by Sema-3A as well as by Npn-1 or Plxn-A2 silencing. Sema-3A-induced axonal retraction appears to be mediated by Fyn Tyrosine Kinase signal and it is associated to the activation of a neuroinflammatory pathway.

Conclusions: These findings indicate that a Sema-3A insult, during the very early stages of neuronal development, affects NP differentiation and connectivity and promotes neuroinflammatory pathways activation.
Rescuing neural cell survival and maturation in a primary autosomal recessive microcephaly-17 (MCPH17) mouse model: effects of the postnatal N-acetyl cysteine treatment

Maryam Khastkhodaei Ardakani¹, Cecilia Astigiano³, Francesco Ferrini², Chiara La Rosa³, Roberta Schellino¹, Marina Boido¹, Serena Bovetti³, Annalisa Buffo¹ and Enrica Boda¹

¹Dept. of Neuroscience and Neuroscience Institute Cavalieri Ottolenghi, Università degli Studi di Torino, Torino, Italy; ²Dept. of Veterinary Sciences, Università degli Studi di Torino, Torino, Italy; ³Dept. of Life Sciences and Systems Biology and Neuroscience Institute Cavalieri Ottolenghi, Università degli Studi di Torino, Torino, Italy

Introduction and objectives: Microcephaly 17 (MCPH17) is a rare neurodevelopmental disorder caused by mutations in the CIT gene, which encodes for the Citron Kinase (CIT-K) protein involved in DNA repair and cytoskeletal dynamics. Patients show reduced brain volume, lissencephaly or simplified gyrification, intellectual disability, motor deficits, epilepsy, and early mortality. Cit-k KO mice recapitulate the human MCPH17 phenotype and shows epilepsy, ataxia and early lethality, as well as DNA damage and reactive oxygen species (ROS) accumulation, apoptosis and maturation defects in neuronal and glial progenitors, and microglia increase. With the aim to identify pharmacological treatments that can reduce the cellular damage accumulation and improve the functional and histopathological phenotype of Cit-k KO mice, we performed a chronic treatment during the first 2 postnatal weeks with the antioxidant drug N-acetylcysteine (NAC), which is already FDA-/EMA-approved and can pass the blood brain barrier.

Results: NAC treatment reduces brain ROS levels and slightly increases Cit-k KO mouse life span. Nevertheless, treated mice show a significant improvement in motor performances and reduction in myoclonus. Major neuroanatomical defects and reduction of cortical interneurons persisted in the treated Cit-k KO mice. Yet, cortical oligodendrocyte progenitors and astrocytes significantly increased in numbers, while microglia density and morphology were largely normalized by NAC treatment. Interestingly, deposition of perineuronal nets (PNNs; i.e. extracellular matrix structures that enwrap and stabilize neuronal synapses) around cortical interneurons was also significantly rescued by NAC treatment, suggesting the promotion of interneuron maturation. In the periphery, NAC promotes the maturation of the neuromuscular junctions, possibly underlying part of the rescue of Cit-k KO mouse motor phenotype. Patch-clamp recordings and in vivo calcium imaging analyses in the cerebral cortex are ongoing to unveil the functional bases of NAC effects.

Conclusions: Our data suggest that NAC postnatal treatment may be beneficial for the treatment of MCPH17.
ORAL SESSION

Excitability, synaptic transmission, network functions
Introduction and objectives: LIMK1 is a serine/threonine kinase that controls actin cytoskeleton dynamics through the phosphorylation and the inactivation of the actin-depolymerizing factors/cofilin. Actin is the major cytoskeletal protein in most cells, playing a critical role in many cellular morphofunctional mechanisms (Bernard, 2007). In dendritic spines, LIMK1 translates Rho GTPase signals in response to neuronal activity, driving the expansion of actin cytoskeleton and dendritic spine enlargements (sLTP). However, although the role of LIMK1 in sLTP has been demonstrated (Bosch et al., 2014; George et al., 2015), it remains elusive whether LIMK1 activation is sufficient to promote sLTP and, consequently, functional long-term potentiation (LTP). Manipulation of LIMK1 activity with chemogenetic approaches has been scarcely explored as a strategy to steer LTP-associated dendritic spine signaling aimed at identifying its implication in synaptic transmission and plasticity. LIMK1 analogs that can be allosterically controlled by safe unnatural cues offer an unprecedented opportunity to investigate causal links between biochemical signalling and neuronal functions. To this end, we developed a genetically encoded engineered LIMK1 to obtain a chemogenetic manipulation of LIMK1 activity in living cells.

Results: In the engineered constructs, the well-tolerated, clinically approved, rapamycin selectively activates LIMK1 resulting in controlled cofilin phosphorylation and sLTP in CA1 pyramidal neurons of organotypic hippocampal slice cultures. Importantly, rapamycin-mediated activation of engineered LIMK1 induced an activity-independent increment of evoked excitatory postsynaptic currents at CA3-CA1 synapses, indicating that our construct boosts glutamatergic transmission in the hippocampus, suggesting that LIMK1 is not just necessary but also sufficient to induce LTP.

Conclusions: The engineered LIMK1 we developed will be useful to understand better the physiological role of LIMK1 not only in synaptic plasticity but also in various cell biological phenomena, as well as for the development of new genetically encoded therapeutic strategies.
Beyond retigabine: design, synthesis, and pharmacological characterization of a potent and chemically-stable neuronal Kv7 channels activator with anticonvulsant activity

Lidia Carotenuto¹, Francesco Miceli¹, Giulia Baroli¹, Vincenzo Barrese¹, Alessia Bertamini², Michele Manfra³, Nunzio Iraci⁴, Carmine Ostacolo⁵, Pietro Campiglia², Maurizio Taglialatela¹.

¹Division of Pharmacology, Dept. Neuroscience, University of Naples Federico II, Naples, Italy; ²Dept. of Pharmacy, University of Salerno, Salerno, Italy; ³Dept. of Sciences, University of Basilicata, Italy; ⁴Dept. of Chemical, Biological, Pharmacological and Environmental Sciences, University of Messina, Italy; ⁵Dept. of Pharmacy, University of Naples Federico II, Naples, Italy

Introduction and objectives: Activation of neuronal voltage-gated potassium channels formed by Kv7.2 and Kv7.3 subunits is a promising pharmacological strategy for the treatment of diseases in which neuronal hyperexcitability is a relevant pathogenetic factor, including epilepsy, neuropathic pain, and neuropsychiatric disorders (Miceli et al., 2018). Retigabine is the prototypical neuronal Kv7 activator (Barrese et al., 2010); this drug is approved for the treatment of partial onset seizures in adults but is no longer available on the market because of its side effects, including skin discoloration caused by photo-induced dimers accumulation (Clark et al., 2015).

In the present work, the structural determinants of the retigabine-binding site and the photo-induced dimers formation were investigated, leading to the synthesis of a small library of retigabine analogues. This library was screened using a fluorescence-based assay (FluxOR™) and patch-clamp electrophysiology in Kv7.2/Kv7.3-expressing cells.

Results: With this library, we identified compound 69 that was unable to form dimers and was more potent than retigabine in enhancing maximal current and in shifting the voltage-dependence (EC50 2.5±1.8 vs 0.15±0.003μM) of Kv7.2/Kv7.3 channels when investigated with patch-clamp. Electrophysiological experiments on the retigabine-insensitive Kv7.2 W236L mutant channels confirmed that CP-69 shares the same binding site of retigabine.

In addition, CP-69 showed higher brain/plasma ratio than retigabine (39.3 vs 2.4), with longer half-life (16h vs 3h) after intraperitoneal (i.p. 1mg/kg) administration in rats. Finally, i.p. administration in mice of CP-69 reduced the severity and increased the latency to the maximal seizure induced in mice by pentylentetrazol (i.p.100mg/kg), at lower doses when compared to retigabine (0.3mg/kg vs 3mg/kg).

Conclusions: In conclusion, CP-69 shows an improved pharmacological profile with respect to retigabine, being therefore a promising candidate for further development as an antiepileptic drug.
**Functional effects of a new dual adenosine A2A/A2B receptor antagonist on CA1 hippocampal synaptic plasticity or during oxygen glucose deprivation**

**Martina Venturini**¹, Clara Santalmasi¹, Federica Cherchi¹, Lucia Frulloni¹, Daniela Catarzi², Vittoria Colotta², Flavia Varano², Felicita Pedata³, Elisabetta Coppi¹ and Anna M. Pugliese¹

¹Department of Neuroscience, Psychology, Drug Research and Child Health, NEUROFARBA, Section of Pharmacology and Toxicology, University of Florence, Florence, Italy; ²Section of Pharmaceutical and Nutraceutical Sciences, University of Florence, Florence, Italy.

**Introduction and objectives:** Adenosine is a ubiquitous endogenous neuromodulator acting in the central nervous system via four receptor subtypes: A₁, A₂A, A₂B and A₃. The A₂A and A₂B receptors (A₂A Rs, A₂B Rs) are coupled to Gₛ-protein and it is recognized that their activation at hippocampal level inhibits Paired Pulse Facilitation (PPF), a model of short-term synaptic plasticity. The reduction in PPF reflects an increase in presynaptic glutamate release. It is known that the hippocampus is a brain area particularly susceptible to a hypoxic-ischemic insult. During cerebral ischemia, a significant increase in glutamate release is responsible for cellular damage and for the appearance of generalized brain depolarization, known as anoxic depolarization (AD), mostly due to extracellular glutamate overload. It is demonstrated that the selective antagonism of A₂ARs or A₂BRs protects from the irreversible synaptic failure induced by severe oxygen glucose deprivation (OGD) in the CA1 region of hippocampus and prevents or delays AD appearance (Pugliese et al., 2009; Fusco et al., 2018). Therefore, the simultaneous block of A₂ARs/A₂BRs could be advantageous for the treatment of complex pathologies, such as stroke. We evaluated for the first time the effects of the new multitarget A₂AR/A₂BR antagonist, P626, on PPF and AD development induced by severe OGD. Extracellular recordings were performed to monitor synaptic transmission and plasticity in the CA1 region of acutely isolated rat hippocampal slices under normoxic conditions or during 30-minutes OGD.

**Results:** P626 (200 nM) completely antagonized PPF reduction induced by CGS21680 (50 nM) or BAY60-6583 (200 nM), a selective A₂AR and A₂BR agonists, respectively. The appearance of AD induced by 30-minutes OGD was significantly delayed by P626 (400 nM).

**Conclusions:** Data demonstrated the beneficial effect of P626 in preventing OGD-induced CA1 damage and in modulating synaptic plasticity in this brain area, thus representing a new class of neuroprotective drugs in ischemia.
POSTER INDEX
POSTER SESSION
Disorders of the nervous system
P01. Effect of neonatal hypoxic-ischemic brain injury on rat neural stem cells differentiation: ex vivo and in vitro studies

Alastra G.1, Baldassarro V. A.1,3, Ferrazzi F.2, Sannia M.3, Calzà L.2,3,4
1Department of Veterinary Medical Sciences, University of Bologna, Italy; 2IRET Foundation, Ozzano Emilia, Italy; 3Health Science and Technologies Interdepartmental Center for Industrial Research (HST-ICIR), University of Bologna, Italy; 4Department of Pharmacy and Biotechnology, University of Bologna, Italy

Introduction and objectives: Neonatal Hypoxic-Ischemic Encephalopathy (HIE) represents a major cause of death and motor-cognitive disabilities in newborns (Miller et al. 2005). In fact, it affects the perinatal phase, a critical period for the central nervous system development, often resulting in neurological damage sequelae, involving neurons and precursors of the oligodendrocyte (OPC). The aim of this study was to investigate the effect of hypoxic-ischemic (HI) conditions on the lineage specification of neural stem cells (NSCs). Two sets of experiments were performed.

Ex Vivo: cells were isolated from the sub-ventricular zone (SVZ) of rats exposed to HI lesion at P8 (unilateral carotid artery ligation), and cultures prepared from the ipsilateral (ipsi) and contralateral side, compared to sham-operated pups (Baldassarro et al. 2020).

In vitro: cells were isolated from the SVZ of healthy P8 rats and exposed in vitro to oxygen (OD), glucose (GD) or oxygen and glucose (OGD) deprivation models. The readouts for both experiments was the lineage analysis at different time-points, as assessed by immunocytochemistry for cell specific markers.

Results: Ex vivo: neuronal maturation (β-tubulin-IR) is slower in HI-derived cells, both ipsi- and contralaterally to the ligation. The oligodendrocyte lineage specification (NG2-IR) and maturation (MBP-IR) is strongly reduced in cultures derived from the HI-ipsi.

In vitro: experiments confirmed the observed effects of HI, indicating a major contribution of the global ischemic insult (GD and OGD) compared to hypoxia.

Preliminary gene expression analysis performed on ex vivo isolated NSCs and OPCs-enriched spheres indicates a selective regulation of Hif1a in oligodendroglial cells and Pax6-5α in neurons.

Conclusion: We demonstrated that lineage specification of neural stem/progenitor cells is highly affected by the HI insult, both on the neuronal and the oligodendrogial differentiation. Studying the HI-mediated damage on NSCs and OPCs is a fundamental step to understand the impact of this lesion on brain maturation, also to identify new therapeutic targets.
P02. Investigating the role of large microglial extracellular vesicles carrying pathogenic misfolded proteins in Alzheimer’s disease and their interaction with neurons

Battocchio E.1,2, Gabrielli M.1, Verderio C.1
1CNR Institute of Neuroscience, Vedano al Lambro, MB, 20854, Italy; 2School of Medicine and Surgery, Department of Medicine and Surgery, University of Milano-Bicocca, Monza, 20900, Italy

Extracellular vesicles (EVs) represent an important mechanism of cell-to-cell communication in the brain. They are lipid-encased nanoparticles that convey bioactive signals from a donor to specific target cells, influencing their functions. EVs can also transfer misfolded proteins involved in pathology, included beta-amyloid (Aβ) and tau protein in Alzheimer’s disease (AD), a condition characterized also by abnormal microglial activation. I recently contributed to a paper interestingly demonstrating that large (>200 nm) microglial EVs carrying Aβ (Aβ-EVs) are able to propagate synaptic dysfunction, a very early sign of AD, in the mouse brain by moving at the axon surface (Gabrielli et al., 2022). In this study, optical manipulation coupled to time-lapse imaging was employed to study EV-neuron interaction, finding that Aβ-EVs are more prone to motility and are faster compared to control EVs from microglia not exposed to Aβ (ctrl-EVs).

My PhD project aims at investigating the functions of microglial large EVs exposed to tau, the other AD key protein, and the molecular basis of their interaction with neurons. To this purpose microglial primary cultures have been exposed to recombinant tau protein (200nM o/n) and EVs have been isolated from the cell supernatant by differential centrifugation upon ATP stimulation (1mM 30mins). Tunable Resistive Pulse Sensing analysis revealed that tau priming of microglia didn’t affect neither production nor size distribution of tau-EVs vs ctrl-EVs. Calcium imaging experiments on neuronal primary cultures exposed to tau-EVs revealed a neurotoxic effect, similarly to Aβ-EVs (Joshi et al., 2014). However, as opposed to Aβ-EVs, optical tweezers/time-lapse imaging experiments showed no difference in tau-EV adhesion and motion ability compared to ctrl-EVs.

This study will help clarify the role of microglial large EVs and their motion at the neuronal surface in AD pathogenesis, providing also molecular targets for the development of novel therapeutic strategies to hamper the disease progression.
P03. Selective behavioral alterations after acute particulate matter exposure in a presymptomatic Multiple Sclerosis mouse model

Bonato M.1, Montarolo F.2,3, Parolisi R.1, Pandino C.1, Bertolotto A.2, Buffo A.1, Boda E.1
1Dept. of Neuroscience, Università degli Studi di Torino, Torino, Italy; 2Neuroscience Institute Cavalieri Ottolenghi, Orbassano (Torino), Italy; 3Neurobiology Unit, Neurology-CReSM (Regional Referring Center of Multiple Sclerosis), AOU San Luigi Gonzaga, Orbassano (Torino), Italy

Introduction and objectives: Exposure to air pollution, and particularly to particulate matter (PM), has been associated with higher rates of Multiple Sclerosis (MS) relapses and increased neuroinflammation in MS patients, suggesting that PM exposure may contribute to MS exacerbation. To address this issue, we have combined the induction of MOG35-55-induced experimental autoimmune encephalomyelitis (EAE) in mice and PM exposure. To study the effects of both short- and long-term PM exposures, mice were exposed to PM10 at dosages relevant for human exposure either acutely, before the immunization or during the pre-symptomatic phase or chronically for 7 days pre-immunization + 7 days post-immunization.

Results: Both chronic and acute PM10 exposures did not significantly modify the disease course or the neuropathology of EAE mice. Yet, few hours after exposure, EAE mice acutely exposed to PM10 during the pre-symptomatic phase (PM-EAE) showed behavioral alterations - that could not be detected neither in control EAE (Ctrl-EAE) nor in PM-exposed wild-type (PM-WT) mice. Namely, when tested in the Open Field, Elevated Plus Maze and Novel Object Recognition tests, PM-EAE mice showed reduced anxiety and a significant increase in novelty seeking. Stereotypic behaviors (i.e. grooming and rearing) instead did not appear selectively affected in PM-EAE mice. Since the observed behavioral phenotypes are frequently associated with alterations of the dopaminergic neurotransmission, along with neuroinflammation markers, we are now studying whether PM10 exposure in EAE mice is associated with changes in brain dopamine levels or in the expression of genes coding for dopamine receptors/transporters and their dynamics/recycling.

Conclusions: Our data indicate that the PM10 exposure did not alter the EAE course, probably due to the low PM10 dose used to mimic the human exposure. However, acute PM10 exposure selectively induces behavioral changes in EAE mice, possibly interacting with their altered neuroimmune/neurotransmission background.
P04. Opposite effects of chronic kidney disease and mild cognitive impairment on the diffusion of water within the perivascular space

Buonincontri V,1, 2 Viggiano D.1, 2
1 Dipartimento di Salute Mentale, fisica e Medicina Preventiva, Università degli studi della Campania “Luigi Vanvitelli”, Napoli; 2 Dipartimento di Scienze Mediche Traslazionali, Università degli studi della Campania “Luigi Vanvitelli”, Napoli

Introduction and objectives: Recent discoveries demonstrate the existence of a network of extracellular spaces between neurons, glial cells, and capillaries that promote the elimination of soluble molecules from the brain (Jessen, N. A. et al., 2015). This system may be disrupted in and contribute to Alzheimer's disease (AD) (Rasmussen, M. K. et al., 2018). Some studies have shown an association between chronic kidney disease (CKD) and dementia. CKD is a risk factor for dementia (Viggiano et al., 2020). The role of brain waste clearance in patients with kidney disease in this process is unknown. To analyze MCI-CKD patients, we used The Alzheimer's Disease Neuroimaging Initiative (ADNI) that involves cohorts of cognitively normal subjects, subjects with mild cognitive impairment (MCI) among which several patients with CKD stage II-III identifiable from the creatinine values and subjects with Alzheimer. We enrolled 12 CKD patients and pair-matched 12 non-CKD patients comparable for age, gender, and MoCA score. In this study we measured the movement of water molecules in the direction of the perivascular space using the diffusion tensor method (DTI) calculated using the FSL software. This approach is based on calculating a diffusion index named ALPS using the ImageJ software.

Results: Our result shows that MCI is accompanied by a reduction of the extracellular spaces (ALPS index) in both control patients and CKD patients, suggesting a reduction of water diffusion in the perivascular space. In the absence of MCI, CKD led to a small, non-significant reduction of ALPS values compared to non-CKD patients.

Conclusions: MCI is accompanied by a reduction of brain extracellular spaces in CKD patients and non-CKD patients, suggesting a common pathophysiology. CKD led to very small effects on brain extracellular spaces even when MCI was not yet present.
Introduction and objectives: Anorexia Nervosa (AN) is a severe eating disorder characterized by a marked reduction of food intake that leads to a drastic weight loss. It affects primarily adolescent girls and young women, has a low rate of recovery and the highest mortality rates among psychiatric disorders (Keski-Rahkonen et Mustelin, 2016). Although the pathophysiology underlying AN has not been fully elucidated, inflammation appears to be a critical component of its course and progression (Caso et al., 2019; Nilsson et al., 2020). For example, increased serum levels of several pro-inflammatory cytokines, such as interleukin (IL)-1 beta (IL-1β), IL-1 and IL-6 and tumor necrosis factor-alpha (TNF-α), were found in anorexic patients (Solmi et al 2015; Dalton et al 2018). The aim of our study was to use a well-established preclinical experimental model of AN to evaluate the cerebral expression of inflammatory mediators, with particular focus on the prefrontal cortex.

Results: Our preliminary data indicate that the ABA paradigm has an impact on the inflammatory state of the animals. Specifically, we found a reduction of the gene expression of the pro-inflammatory cytokines TNF-α and IL-1βas well as an increase of the gene expression of IL-6. We also noticed a reduced expression of the marker of macrophages CD11B and of NLRP3 inflammasome which is a critical component of the innate immune system that mediates the secretion of proinflammatory cytokines IL-1β in response to cellular damage.

Conclusions: Taken together, these results indicate a general reduction of inflammation in prefrontal cortex of ABA rats, which might represent an initial compensatory response to the ABA paradigm.

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Spinal Muscular Atrophy (SMA) is a motor neuron (MN) pediatric disease caused by a low expression of survival motor neuron (SMN) protein due to the total absence of SMN1 gene that is only partially rescued by the paralogue one SMN2. It determines not only MN loss, but also impairment at the peripheral level (i.e. skeletal muscles and heart) (Shababi et al., 2014). Unfortunately, the available therapies (SMN-dependent), despite their efficacy, show several limitations and the identification of new targets and therapeutic strategies are required.

Recently it has been demonstrated that mitochondria, the “powerhouse of the cells”, undergo both a morphological and a functional “switch” in response to stress (Stanga et al., 2020). Since all the affected tissues in SMA need a lot of energy, mitochondria appear as promising targets for new treatment investigation.

The aims of this project are to identify, through a bioinformatic approach (Molineris et al., 2013), SMN1-anticorrelated mitochondrial genes and to normalize their expression regulating mitochondrial functionality.

We first identified eight strongly SMN1-anticorrelated genes expressed in the most affected tissues in SMA (CNS, muscles and heart): COX7A1, GCSH, BAG1, GOLPH3, DNAJC5, SLC25A36, GLRX2 and UQCRC2. To assess their expression in SMA, we exploited samples (lumbar spinal cord, brain, heart and skeletal muscles) obtained from SMNdelta7 mice (an intermediate model of SMA), sacrificing WT and SMA animals in an early symptomatic stage of the pathology (postnatal day 5, P5), after a period of behavioral testing (P2-P5). RT-PCR on collected tissues revealed in particular a marked upregulation of GCSH in spinal cord, brain and gastrocnemius of SMA mice. Since the preliminary observation of SMN1 and GCSH anticorrelation, we can hypothesize an existing relationship between their expression. Therefore, normalizing GCSH levels could determine a mitochondrial integrity restoration and a recovery from cellular disease impairments.

Bibliography:


The reversible Carnitine Palmitoyltransferase1 inhibitor ST1326 ameliorates the neurodegenerative phenotype in a Drosophila Huntington model by acting on the expression of carnitine-related genes

Carillo M. R., Bertapelle C., Peluso G., Digilio F.A
National Research Council, Napoli, Italy and University of Campania “Luigi Vanvitelli”

Background: Huntington’s Disease (HD) is a dramatic neurodegenerative disorder caused by the abnormal expansion of a CAG triplet in the huntingtin gene, producing an aberrant protein. Because it results in death of neurons in the cortex, the patients primarily present with neurological symptoms, but recently metabolic changes resulting from mitochondrial dysfunction have been identified as novel pathological features. The carnitine shuttle is a complex consisting of three enzymes whose function is to transport the long-chain fatty acids into the mitochondria. Here, its pharmacological modification was used to test the hypothesis that shifting metabolism to lipid oxidation exacerbates the HD symptoms. Behavioral and transcriptional analyses were performed with the fruit fly Drosophila melanogaster was used to perform to test the involvement of the carnitine cycle in this pathogenesis. This revealed that, pharmacological inhibition of CPT1, the rate-limiting enzyme of the carnitine cycle, ameliorates the HD symptoms, likely acting on the expression of carnitine-related genes.

Objective: test the involvement of the carnitine cycle in the Huntington disease.

Methods: Pharmacological inhibition of one of the major components of the carnitine shuttle (CPT1)

Results: the link between Huntington’s neurodegeneration and metabolic effects is still not clear, but therapeutic strategies that affect mitochondrial energy processes using small compounds could help in the development of appropriate treatments to slow the progression of the disease.
P08. Curcumin prevents oxidative stress induced alteration on ARPE-19 cells

Carozza G.1, Tisi A.1, Flati V.1, Feligioni M.2, Maccarone R.1
1Department of Biotechnological and Applied Clinical Sciences, University of L’Aquila, 67100-L’Aquila; 2European Brain Research Institute, 00161 Rome, Italy

Introduction and objectives: Oxidative stress is a major risk factor in the pathogenesis of Age-Related Macular Degeneration (AMD), the leading cause of blindness worldwide in the elderly. Retinal structures are particularly vulnerable to oxidative stress due to their high rate of metabolism and with aging there is an impairment of antioxidant defenses, contributing to degenerative events of AMD (Tisi et al., 2020). The main goal of this research was to find an effective strategy to counteract the degenerative effects of oxidative stress on the RPE cells.

Results: For this purpose, we tested Curcumin, a natural compound with well-known antioxidant, antiapoptotic and anti-inflammatory properties, in in vitro experiments on ARPE-19 cells. Three different concentrations of curcumin (0,01mM; 0,05mM and 0,1mM) were added to the culture medium and oxidative stress was induced by H₂O₂ supplementation. Cell viability was evaluated by Crystal Violet assay, showing curcumin capability to counteract H₂O₂-induced cell death in ARPE-19 cells. In addition, the higher concentration of curcumin (0,05mM and 0,1mM) blocked cell proliferation as highlighted by CCK-8 assay. Our data also showed that curcumin is able to down-regulate the expression of two important autophagic markers such as LC3BII and p62. Moreover, curcumin treatment induces evident morphological changes in ARPE-19 cells which acquire a neuronal-like phenotype. Accordingly, Curcumin-treated cells resulted to be immunoreactive for Anti-Neurofilament through immunofluorescence.

Conclusions: Taken together, our data suggest a protective effect of curcumin against oxidative stress, and also showed new interesting evidences that pave the way for further investigations.
P09. Survival of VTA dopaminergic neurons is associated with overexpression of Ca\(^{2+}\)-binding proteins in the Tg2576 mouse model of Alzheimer’s Disease

Cauzzi E.
Laboratory of Molecular Neuroscience, Campus Bio-medico University of Rome, Italy; Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy; Institute of Biochemistry and Cell Biology (IBBC) - National Research Council (CNR), Rome, Italy

Introduction and objectives: recent clinical and experimental studies have highlighted the involvement of Ventral Tegmental Area (VTA) dopaminergic (DA) neurons for the early pathogenesis of Alzheimer’s Disease (AD) (Bozzali et al., 2019; Cordella et al., 2018; D’Amelio et al., 2018; De Marco and Venneri, 2018; Iaccarino et al., 2020; Nobili et al., 2017; Sala et al., 2021; Serra et al., 2018, 2021). We have previously described a progressive and selective degeneration of these neurons in the Tg2576 mouse model of AD, long before amyloid-beta plaques formation (Cordella et al., 2018; Nobili et al., 2017; Spoleti et al., 2022). The degenerative process in DA neurons is associated with an autophagy flux impairment, whose rescue can prevent neurodegeneration (La Barbera et al., 2021). Alterations in autophagy may be the basis for accumulation of damaged mitochondria, leading to impairments in Ca\(^{2+}\) homeostasis (Cali et al., 2012; Müller et al., 2018), and to functional and structural deterioration of DA neurons. To investigate these aspects in Tg2576 mice, we focused on DA neurons projecting to the Nucleus Accumbens (NAc), a major component of the ventral striatum, precociously affected in AD patients.

Results: We demonstrate that DA neurons accumulate damaged mitochondria, while Apoptosis-inducing factor (AIF), released from mitochondria, translocates to the nucleus. We show an age-dependent loss of DA neurons from the VTA, and importantly we found an alteration in Ca\(^{2+}\) homeostasis in surviving DA neurons. Particularly, we observed that surviving neurons upregulate Ca\(^{2+}\)-binding proteins while the free cytosolic levels of Ca\(^{2+}\) are decreased in the same DA neurons of Tg2576 mice.

Conclusions: Ca\(^{2+}\)-binding proteins overexpression in VTA DA neurons may help cells to survive the neurodegeneration, increasing their ability to buffer free Ca\(^{2+}\). Thus, exploring strategies to overexpress Ca\(^{2+}\)-binding proteins could be fundamental to reduce neuronal suffering and improve memory and cognition in AD.
P10. Alternative Translation Initiation as a novel strategy to block toxicity of the mutant Androgen Receptor in SBMA

Chierichetti M.¹, Cristofani R.¹, Rusmini P.¹, Ferrari V.¹, Tedesco B.¹, Cozzi M.¹, Casarotto E.¹, Mina F.¹, Pramaggiore P.¹, Crippa V.¹, Galbiati M.¹, Piccolella M.¹, Poletti A.¹

¹Dipartimento di Scienze Farmacologiche e Biomolecolari-Centre of Excellence on Neurodegenerative Diseases, Università degli Studi di Milano, Italia

Introduction and objectives: Spinal and Bulbar Muscular Atrophy is a neurodegenerative disease linked to a CAG repeat expansion in the Androgen Receptor (AR) gene, which is translated into a polyglutamine tract in the AR N-terminus leading to aggregation after androgen binding and dimerization (La Spada et al., 1991). Different start codons (AUGs) are involved in AR translation. I-AUG leads to transcription of a full-length AR (AR-B) which includes the pathogenic polyQ tract in SBMA. II-AUG is an alternative AUG leading to the transcription of isoform AR-A, which does not contain the polyQ tract (Jenster et al., 1995).

Current therapeutic approaches are based on androgen blockage and/or downregulation of AR, which causes undesired endocrine side effects. For this reason, we aim to develop an effective strategy to selectively drive the AR translation from the II-AUG via antisense oligonucleotide (ASO) and a library of FDA approved drugs.

Results: First of all, we demonstrated that AR-A and AR-B have similar expression levels in transiently transfected cells. After that, we demonstrated that AR aggregates are not present in cells expressing AR-A. We also compared the transactivation activity of AR-A through luciferase assay, and we found that AR-A had a lower transactivation capability compared to AR-B, but similar to that of ARpolyQ.

We have also developed a double report screening vector designed to detect different AR isoforms expression in relation to the signal obtained. Due to the complexity of the plasmid, we have performed aggregation propensity and protein solubility study, to obtain a suitable plasmid to produce a stable transfected cell line. This cell line has been sorted to obtain a homogenous population to perform ASO and drugs screening.

Conclusions: Those data suggest that alternative translation induction of AR-A could be a possible therapeutic approach due to its biologic behaviour such as transactivation capability and absence of aggregates formation.

Bibliography:

P11. Synaptic alterations in the auditory cortex and hippocampus underlie social deficits in the Synapsin II knockout mouse

Ciano L. 1,2, Esposito A. 1,2, Franchi F. 1,2, Fassio A. 2,3, Michetti C. 1,2, Benfenati F. 1,3
1Center for Synaptic Neuroscience and Technology, Italian Institute of Technology, Genova, Italy; 2Department of Experimental Medicine, University of Genova, Genova, Italy; 3IRCCS, Policlinico San Martino Hospital, Genova, Italy.

Introduction and objectives: Autism spectrum disorders (ASDs) are heterogeneous neurodevelopmental disorders characterized by two main symptoms: social deficits and repetitive behaviors (DSM-V, 2013). Autistic children often exhibit several secondary symptoms including epilepsy (Levy et al., 2010; Fassio et al., 2011 a; Woolfenden et al., 2012). Mutations in the Synapsin2 (SYN2) gene, associated with ASD and epilepsy in humans, are causative for an imbalance between excitatory and inhibitory systems (Fassio et al., 2011 b; Corradi et al., 2014). Synapsins are a family of neuron-specific phosphoproteins which control synaptic vesicle trafficking and modulate neurotransmitter release at the presynaptic terminal (Cesca et al., 2010). Mice lacking SYN2 (SynII KO) display autistic-like traits with a strong reduction of ultrasonic vocalizations (USV) and social sniffing, repetitive behaviors (self-grooming) and mild cognitive impairments (deficit in social memory and recognition) (Greco et al., 2013; Michetti et al., 2017). Furthermore, SynII KO mice display epileptic seizures that appear at 2-3 months of age (Etholm et al., 2012). Interestingly, we recently showed that the impaired USV phenotype strongly correlates with a reduced functional connectivity in the auditory cortex and hippocampus, two brain regions playing an important role in social behavior (Michetti et al., 2017). Our goal was to clarify how synaptic alterations in these regions impact on the social brain circuitry generating pathological conditions. To this aim we characterized the synaptic alterations in the auditory cortex and hippocampus using western blotting analysis and immunohistochemistry.

Results: Our results show a significant reduction in presynaptic and postsynaptic markers of the GABAergic system together with alterations in synaptic density in these areas of SynII KO mice.

Conclusions: These results indicate that developmental changes in the neural connectivity of specific cortical areas may underlie the epileptic and social phenotype of SynII KO mice.
P12. Epigenetic and transcriptional perturbations underlie defective myeloarchitecture in the visual cortex of CDKL5-deficiency disorder patients

Comai D.1, Pizzo R.1, Devi S.1, Lauria A.2,3, Anselmi F.2,3, Gurgone A.1, Oliviero S.2,3, Giustetto M.1
1Department of Neuroscience, University of Turin, Turin (Italy); 2Department of Life Sciences and System Biology and MBC, University of Turin, Turin (Italy); 3IIGM - Italian Institute for Genomic Medicine, Candiolo (Italy)

Introduction and objectives: Cyclin-dependent kinase-like 5 (CDKL5) deficiency disorder (CDD) is a rare and debilitating neurodevelopmental disease resulting from mutations of the X-linked CDKL5 gene (Lin et al., 2005). Studies in preclinical models revealed that CDKL5 encodes a serine/threonine kinase involved in dendritic spines stabilization (Della Sala et al., 2016; Pizzo et al., 2016) and cytoskeleton dynamics (Baltussen et al., 2018) in neurons. Moreover, CDKL5 interactions with epigenetic factors (MeCP2, DNMT1 and HDAC4) (Mari et al., 2005; Kameshita et al., 2008; Trazzi et al., 2014) have also been disclosed, suggesting that CDKL5 may participate in the regulation of gene expression. Currently, very little is known on CDKL5 role in the human brain. To start filling this gap, we investigated both epigenetic marks and transcriptional signatures in post-mortem primary visual cortex (BA17) samples from two CDD patients and two healthy age- and sex-matched controls.

Results: Immunofluorescence and reduced representation bisulfite sequencing (RRBS) techniques revealed that BA17 of CDD patients shows extensive alterations in both histones’ modifications and DNA methylation. These epigenetic abnormalities are paralleled by altered expression levels of genes, mostly involved in the onset and/or maintenance of the myelination process. Additionally, integrative analysis of dysregulated genes in CDD and other clinically-related neurodevelopmental disorders (i.e. Rett syndrome and autism spectrum disorder), showed a significant overlap between the datasets, suggesting that these pathologies share altered biological processes. Finally, we find that the myeloarchitecture in CDD patients seems severely altered, a cellular phenotype that is also present in CDKL5-KO mice.

Conclusions: This study, even though generated on a limited number of samples, discloses molecular and cellular signatures pointing to abnormal cortical myelination in CDD. Our findings support the idea to exploit white matter organization in human patients as a promising biomarker for the disease.
Introduction and objectives: Intellectual disability (ID) is a neurodevelopmental pathological condition characterised by limitations in intellectual functioning and adaptive behaviour. The Bosch-Boonstra-Schaaf optic atrophy-intellectual syndrome (BBSOAS; OMIN#615722), is a rare disorder caused by mutations in the NR2F1 gene, characterized by ID associated to global developmental delay, optic nerve atrophy, hypotonia, seizure and autistic traits. The transcriptional regulator Nr2f1 (i.e., COUP-TFI) is a key player in multiple cellular processes during brain development (Tocco et al., 2021). Interestingly, alterations in postnatal hippocampal neurogenesis have been reported in animal models of ID and recent findings suggest that a deficit in hippocampal plasticity may contribute to BBSOAS (Chen et al., 2020). Here, to investigate possible effects of Nr2f1 haploinsufficiency on the hippocampal circuit we took advantage of a recently validated BBSOAS mouse model (i.e., constitutive Nr2f1-heterozygous mice) and focussed on the adult hippocampal dentate gyrus (DG).

Results: Although, no differences were found in the density of newly generated doublecortin (DCX)-positive immature neurons, data from 3D morphometric reconstruction strongly suggests that Nr2f1 haploinsufficiency influences dendritic architecture and proper development of adult-born DG neurons, leading to the appearance of atypical and peculiar neuronal morphologies that are usually associated with pathological conditions and aberrant hippocampal circuitry rearrangements. Interestingly, preliminary data on the expression of the neuronal activity-dependent gene c-fos suggest an altered recruitment of neuronal ensemble in the DG of Nr2f1-heterozygous mice.

Conclusions: Our data started to unravel morphological and functional alterations in the DG of adult Nr2f1-heterozygous mice. Future investigations will be aimed at understanding the underlying mechanisms and particularly the cell-intrinsic versus cell-extrinsic components of the observed defects.

Bibliography:


Adult neural stem cells (NSCs) reside in the subventricular zone (SVZ) of the lateral ventricles and in the subgranular zone (SGZ) of the dentate gyrus. These brain areas act as neurogenic niches thanks to a heterogeneous and specialized micro-environment that promotes and sustains adult neurogenesis (Seri et al 2001). During aging, changes to the niche micro-environment progressively reduce NSC ability to generate neurons. Our group identified Dbx2 (Developing brain homeobox gene 2) as a candidate regulator of age-associated neurogenic decline in the mouse SVZ (Lupo et al 2018). Of note, Dbx2 is also expressed in astrocytes and regulates the expression of some genes important for their maturation (Lattke et al 2021), but the role of Dbx2 in astrocytes functions remains to be fully elucidated.

Astrocytes are an important component of the NSC niche, contributing to the establishment and maintenance of a permissive neurogenic environment. However, during physiological aging processes, astrocytes undergo age-associated changes (Boisvert et al 2018) that might impair NSC properties and neuron generation. In this study we intend to investigate the role of Dbx2 in the regulation of astrocyte functions.

NSCs derived from the murine adult SVZ were engineered with an inducible expression cassette, allowing for overexpression of Dbx2 by the administration of doxycycline (Dox). NSCs were first differentiated into astrocyte-like cells and then treated with or without Dox for 24h or 48h. We have been carrying out gene expression analyses to define the identity and proprieties of Dbx2-overexpressing astrocytes at molecular level. Furthermore, to assess astrocyte functional properties, we have been collecting conditioned media (CM) from control (-Dox) or Dbx2-overexpressing (+Dox) astrocyte cultures and testing their effects on undifferentiated NSCs. CM collected from astrocytes +Dox inhibits NSC differentiation into neurons when compared with CM -Dox. These preliminary data suggest that increased expression levels of Dbx2 change astrocyte properties, shaping their function toward an anti-neurogenic phenotype.
P15. Chemogenetic manipulations of hippocampus excitability in Ambra1+/− mice with sexual dimorphism of autistic traits: implications for female autism

De Introna M.1,2,3, Sabetta A.3, Nobili A.3,4, Stabile F.3, D’Addario S. L.3,5, Ventura R.3,5, Ammassari-Teule M.3,6, Pignataro A.2,3

1Department of Systems Medicine, Tor Vergata University, Rome, Italy; 2Institute of Translational Pharmacology, CNR-National Research Council, Rome, Italy; 3IRCCS Santa Lucia Foundation (FSL), Centro di Ricerca Europeo sul Cervello (CERC), Rome, Italy; 4University Campus Bio-Medico, Rome, Italy; 5Department of Psychology, University Sapienza, Rome, Italy; 6Institute of Biochemistry and Cell Biology, CNR National Research Council, Rome, Italy

Introduction and objectives: Haploinsufficiency for the proautophagic AMBRA1 gene causes a reduction in the number of hippocampal inhibitory parvalbuminergic interneurons (PV-IN), increases hippocampus excitability, and triggers an autistic phenotype in the female mice whereas males are insensitive to diminished AMBRA1 expression [1,2]. We sought to determine whether these hippocampal dysregulations are causally involved - and specific of - this model of female autism, and which factors make it they are observed in this specific gender x mutation condition.

We used excitatory DREADDs to increase PV+ interneurons activity, decrease hippocampal excitability, and rescue the autistic phenotype of PV-Cre Ambra1+/− female mice. We used inhibitory DREADDs to decrease PV+ interneuron activity, increase hippocampal excitability, and trigger an autistic phenotype in non-autistic PV_Cre Ambra1+/− males and Wt_Cre mice of both genders. Hypothesizing that sex steroid hormones involved in the female protective effect (FPE) could be altered in Ambra1+/− females, we measured hippocampal estrogens receptors levels in each mutation x gender condition.

Results: Downregulation of hippocampal hyperexcitability in autistic Ambra1+/− females rescued social and attentional impairments, and dendritic spines alterations. Opposite chemogenetic manipulations triggered an autistic phenotype in all groups of non-autistic mice. Estrogen receptors were selectively reduced in Ambra1+/− females compared to wild-type females.

Conclusions: Hippocampus hyperexcitability is a sufficient condition to observe autistic traits without gender or mutation alone being necessary conditions. Combined decrease of autophagy and hippocampal estrogens represents a major risk to female autism. Uncovering that autophagy genes modulate expression of ERs might have important therapeutic implications for autistic females.

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**P16. Correlation between D-loop methylation level and mtDNA copy number in Aicardi-Goutières patients**

**Dragoni F.**\(^1\)\(^,2\), Garau J.\(^1\), Orcesi S.\(^1\), Pansarasa O.\(^1\), Gagliardi S.\(^1\)

\(^1\)IRCCS Mondino Foundation, Pavia, Italy; \(^2\)Department of Biology and Biotechnology “Lazzaro Spallanzani”, University of Pavia, Pavia, Italy

**Introduction and objectives:** Aicardi-Goutières Syndrome (AGS) is a pediatric rare disorder that affects the brain, the immune system and the skin. Mutations in 9 AGS genes lead to an accumulation of endogenous nucleic acids (NAs) which are recognized as foreign NAs of viral origin by the organism triggering an abnormal Interferon-alpha (IFN-α) mediated immune response. Mitochondrial dysfunction may lead to the release of mtDNA and trigger immunological pathways with the production of IFN-α. Alterations in methylation levels of the mitochondrial displacement loop (D-loop) region, which governs mtDNA replication, were recently discovered in other neurological disorders, i.e. Alzheimer’s disease and amyotrophic lateral sclerosis (Stoccoro et al., 2020, Stoccoro et al., 2021). To date, nothing is known about D-loop region methylation levels in AGS patients. Aim of this study was to investigate the D-loop methylation levels and the mtDNA copy number in peripheral blood of AGS patients and healthy controls. Pyrosequencing analysis of D-loop methylation levels and quantitative analysis of mtDNA copy number were performed in peripheral blood cells from 25 AGS patients and 22 age- and sex-matched controls.

**Results:** D-loop methylation levels were significantly higher in AGS patients compared to controls, and the difference was driven by the \textit{RNASEH2B} A177T mutated patients. Also, the mtDNA copy number was considerably greater in AGS patients, with the \textit{RNASEH2B} mutant individuals driving the difference. A positive correlation was detected between mtDNA copy number and D-Loop methylation levels in total samples, controls and AGS patients. Moreover, a significant positive correlation was detected between D-Loop methylation of controls group, mtDNA copy number of AGS patients and age of sampling.

**Conclusions:** These findings suggest that D-loop methylation levels and mitochondrial replication are intimately linked, and that could contribute, as compensatory mechanisms, to mitochondrial dysfunction.
P17. Increased DUX4 expression and correlation with TDP-43 in peripheral cells from ALS patients
Duranti E.1,5 Sala G.1, D’Orlando C.1, Gerardi F.2, Riva N.3, Lunetta C.2, Meneveri R.1, Ferrarese C.1,4, Tremolizzo L.1,4
1School of Medicine and Surgery and Milan Center for Neuroscience (NeuroMI), University of Milano-Bicocca, Monza; 2NEuroMuscular Omnicentre (NEMO), Fondazione Serena Onlus, Milano; 3Neurology Unit, IRCCS San Raffaele Scientific Institute Milano; 4Department of Neurology, ASST Monza, San Gerardo Hospital, Monza; 5PhD program in Neuroscience, University of Milano-Bicocca, Monza

Introduction and objectives: Amyotrophic Lateral Sclerosis (ALS) is a progressive fatal neuromuscular disease characterized by selective motor neurons loss. TDP-43 (TAR DNA–binding protein 43) is an important component of aggregates found in the cytoplasm of motor neurons of ALS patients and in muscle cells of patients with Facioscapulohumeral dystrophy (FSHD), a disease caused by the aberrant expression of DUX4 protein. Interestingly, it has been demonstrated that the expression of DUX4 is able to induce aggregation of TDP-43 in human FSHD myoblasts as well as in healthy cells transfected with DUX4 (Homma et al., 2014). Unpublished data from our laboratory showed that DUX4 is expressed in human peripheral blood mononuclear cells (PBMCs) and, based on these premises, we decided to study the expression of DUX4 in PBMCs from ALS patients and to verify the existence of a possible correlation with TDP43 expression and aggregation.

Results: The analysis of DUX4 gene and protein expression showed a 3-fold increase (p<0.0001) of DUX4 mRNA and protein levels in PBMCs form ALS patients (N=46) with respect to controls (N=15). According to previous results from our group (Arosio et al., 2019), we confirmed an increase of TDP-43 protein levels in ALS PBMCs, and we also identified an increase of the truncated forms TDP-35 and TDP-25. A positive correlation between DUX4 and TDP-43 (r=0.61, p<0.0001), TDP-35 (r=0.48, p<0.001) or TDP-25 (r=0.46, p<0.005) protein levels was found in ALS samples. In addition, immunofluorescence analyses performed in a subgroup of ALS PBMCs, indicated that, while control cells showed a low DUX4 expression at the nuclear level, PBMCs from ALS patients showed an increased DUX4 expression and a mis-localization in the perinuclear and cytoplasmic areas. Interestingly, in ALS samples DUX4 co-localized with TDP-43 in the perinuclear region and showed a tendency to aggregate. Preliminary results from ongoing experiments performed in skin fibroblasts obtained from ALS patients indicated an increased DUX4 expression also in this cell type. Finally, we demonstrated in an in vitro model that DUX4 upregulation induced an increase of insoluble TDP-43.

Conclusion: These results indicate that DUX4 is increased in ALS cells and suggest a possible role for DUX4 in TDP-43 accumulation and aggregation, although further studies are needed to clarify the underlying mechanisms.

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Introduction and objectives: Dopamine is a key modulatory catecholamine neurotransmitter that regulates the control of voluntary movements through the nigrostriatal pathway. At the striatum, dopamine exerts its action upon binding to metabotropic receptors subdivided into two families (D1- or D2-type) according to the class of G proteins to which they are coupled (stimulatory or inhibitory, respectively). Although the pathophysiological role of dopamine as a neurotransmitter is well-described, its influence on non-neuronal glial cells remains poorly understood. Among them, astrocytes and microglia have been reported to express dopamine receptors (Mastroeni et al., 2009) and to respond to dopamine (Corkrum and Araque, 2021). Astrocytes play critical roles in the brain and are required for neuronal development and survival, such that astrocyte impairment has been found to contribute to neuronal dysfunction in several neurodegenerative diseases (Phatnani et al., 2015). Accordingly, striatal astrocytes are important for maintaining the integrity of the nigrostriatal dopaminergic system, but the precise role of dopamine signaling on astrocyte functions is still unknown.

Results: Our preliminary data suggest that primary astrocytes isolated from mouse striatum express higher levels of dopamine receptors as compared to astrocytes derived from other brain subregions. Moreover, striatal astrocytes are able to respond to dopamine in the low nanomolar range, which is reported to correspond to the concentration that elicits D2-type receptor-mediated responses. More specifically, we observed the activation of both canonical G protein-coupled and alternative β-arrestin2-dependent (Klein et al., 2019) signaling pathways downstream to D2-type receptors.

Conclusions: Based on these indications, our data are crucial to elucidate the physiological role of dopamine as a modulator of non-neuronal astrocytic function. Since the depletion of striatal dopamine content is a major contributor to the motor symptoms observed in Parkinson’s disease, this study is key to understanding whether dopamine loss-related astrocyte dysfunctions may represent an early event in the disease progression.
P19. Adenosine A2B receptor activation regulates oligodendroglial differentiation and myelination: an in vitro study
Frulloni L. 1 Santalmasi C. 1, Cherchi F. 1, Venturini M. 1, Magni G. 2, Rossi F. 2, Pedata F. 1, Cencetti F. 3, Coppi E. 1, Pugliese A.M. 1
1 Department of Neuroscience, Psychology, Drug Research and Child Health, NEUROFARBA, Section of Pharmacology and Toxicology, University of Florence, Florence, Italy; 2 Istituto di Fisica Applicata, CNR, Via Madonna del Piano 10, Sesto Fiorentino 50019, Italy; 3 Department of Experimental and Clinical Biomedical Sciences, University of Florence, Florence, Italy

Introduction and objectives: Oligodendrocyte-formed myelin sheaths allow fast synaptic transmission in the brain and their degeneration leads to demyelinating diseases, such as multiple sclerosis (MS). Remyelination requires the differentiation of oligodendrocyte progenitor cells (OPCs) into mature oligodendrocytes (OLs). Adenosine and its receptors (A1, A2A, A2B and A3 receptors: A1R, A2AR, A2BR and A3R) are crucial mediators in remyelination processes. Remarkably, A1Rs facilitate OPC maturation (Stevens et al., 2002) and migration (Othman et al., 2003), whereas the A3R initiates apoptosis in OPCs (Gonzalez-Fernandez et al., 2014). We contribute to demonstrate that selective stimulation of A2ARs and A2BRs decrease OPC maturation by inhibiting potassium currents necessary to their differentiation (Coppi et al., 2013, 2020). In this work we further explored the functional role of A2BRs in modulating potassium currents and OPC maturation in cultured OPCs. In addition, we characterized their involvement on myelination by using dorsal root ganglion (DRG) neurons/OPC co-cultures. Patch-clamp recordings coupled to quantitative Real-Time PCR and immunocytochemistry were used.

Results: We confirmed that BAY60-6583, a selective A2BR agonist, inhibited potassium currents in rat OPCs and reduced their differentiation, reported as the decrease in MBP/MAG gene expression. Surprisingly, BAY60-6583 increased axonal myelination in DRG/OPC co-cultures and enhanced action potential firing in primary DRG cultures.

Conclusions: Our data confirm that A2BR activation prevents OPC differentiation, by inhibiting potassium currents. However, in OPC-DRG co-cultures A2BRs promote axonal myelination, probably by enhancing action potential firing in DRG neurons. These results suggest that activation of A2BRs modulates different functions in oligodendrogliogenesis, depending on their cellular localization and may represent a valuable target in demyelinating pathologies, such as MS.
P20. Deficient NF-κB/c-Rel activity in pathophysiology of Parkinson’s disease

Gennari M.M.1, Porrini V.1, Parrella E.1, Gussago C.1, Pilotto A2, Vezzoli M.1, Bellucci A.1, Padovani A.2, Pizzi M.1

1Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy; 2Neurology Unit, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy

Introduction and objectives: The loss of dopaminergic neurons in the substantia nigra (SN) pars compacta and the accumulation of misfolded α-synuclein (α-syn) are considered the pathological hallmarks of Parkinson’s disease (PD). We previously reported that NF-κB/c-Rel deficient mice develop a late-onset parkinsonism, encompassing nigrostriatal degeneration, L-DOPA-reversible hypomotility and caudal-rostral α-syn deposition (Baiguera et al., 2012; Parrella et al., 2019). To assess whether c-Rel dysregulation can be implied in PD pathophysiology, we investigate c-Rel DNA-binding activity in both SN and peripheral blood mononuclear cells (PBMCs) of healthy controls (HC) and PD patients.

Results: DNA-based ELISA revealed a significant reduction in c-Rel activity in both post-mortem SN and PBMCs from PD patients when compared with age-matched HC ones, although no differences in c-Rel protein level were observed. c-Rel DNA-binding activity has been shown to be affected by post-translational modifications (PTMs) such as O-linked-β-N-acetylglucosamine (O-GlcNAc) (Ramakrishnan et al., 2013). Interestingly, altered O-GlcNAc glycosylation (O-GlcNacylation) have been found in multiple neurodegeneration-related pathway (Balana and Pratt, 2021). To assess the impact of O-GlcNacylation on c-Rel DNA-binding capability, cultured PBMCs from HC and PD patients were exposed to 11 mM glucose (not stimulated) or 30 mM glucose and 0.1 mM PUGNAc (stimulated) in order to increase the extent of total O-GlcNacylation. Stimulated HC PBMCs displayed a significant increase in c-Rel DNA-binding activity when compared with not stimulated ones, while we didn’t see any marked differences in PD patients PBMCs. Finally, an increase in total O-GlcNAc levels was only recorded in HC PBMCs when exposed to high level of glucose and PUGNAc. These results are functional to our ongoing studies on c-Rel O-GlcNacylation state in PD.

Conclusions: Our findings suggest that c-Rel dysregulation is implied in the pathophysiology of PD, whereas an altered O-GlcNacylation state could explain the defect in c-Rel DNA-binding activity.
P21. New evidence of a different activation of astrocytes in LPS and MCAO gliosis mice model: a computational transcriptome analysis

Gioia C., Goglia I., Martorana F., Bertolazzi P., Colangelo A.M.

1Laboratory of Neuroscience “R. Levi-Montalcini”, Dept. of Biotechnology and Biosciences, University of Milano-Bicocca, Milano, Italy; 2PhD course in Translational and Molecular Medicine (DIMET), University of Milano-Bicocca, Milano, Italy; 3PhD program in Neuroscience, School of Medicine and surgery, University of Milano-Bicocca, Milano, Italy; 4Institute of Systems Analysis and Computer Science A. Ruberti (IASI), National Research Council (CNR), Via dei Taurini 19, 00185 Rome, Italy

Introduction and objectives: Reactive astrogliosis is a response of astrocytes to brain disease and injuries as ischemia, neurodegeneration and infection. Studies suggest a possible polarization of astrocytes induced by different stimuli. A classification in two phenotype was proposed: A1 after LPS stimuli with a detrimental phenotype and A2 after ischemia with neuroprotective effects. However, this simple classification does not summarize the heterogeneity of astrocytic responses. To better understand the divergence between LPS and ischemic stimuli, we analyzed gene expression data (from GEO database, GSE35338) of astrocytes taken from LPS-treated mice and mice with focal ischemic stroke produced by transient middle cerebral artery occlusion (MCAO) (Zamanian JL et al., 2012). The authors of this study conducted a transcriptome analysis which led to the identification of a list of induced genes and upregulated pathways.

Results: We conducted a different analysis using a gene set analysis method (GSEA), which allowed us to identify pathway alterations capable of adding new information on phenotypic differences in the two gliosis status. Data suggest a different upregulation of nucleotide metabolism pathways, i.e., pathways concerning nucleotide catabolism and biosynthesis are respectively upregulated in the LPS and MCAO groups. Instead, TCA cycle and respiratory electron transport chain pathways are significantly downregulated at day-1 after MCAO, in contrast to what occurs in the LPS astrocytes groups and MCAO at day-3. Moreover, upregulation of biosynthesis of specialized pro-resolving mediators (Tiberi M, 2021) in MCAO groups suggest that reactive astrocytes in ischemia exhibit a protective phenotype. Finally, analysis suggests that specific DNA repair (base excision repair and DNA double-strand break repair) and cell cycle pathways are upregulated predominantly after MCAO at day-3.

Conclusions: This study suggests new possible aspects of the differential activation of astrocytes after different stimuli and how different computational approaches may be helpful in retrieving new information from experimental data.

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P22. Generation and characterization of the c19orf12 mutant zebrafish model

Gnutti B., Agazzi P., Mignani L., Canino B.F., Zizioli D., Borsani G., Finazzi D.
Department of Molecular and Translational Medicine, University of Brescia, Italy

**Introduction and objectives:** Mutations in the orphan gene C19orf12 cause Mitochondrial membrane Protein Associated Neurodegeneration (MPAN), a rare inherited neurologic disorder, belonging to the Neurodegeneration with Brain Iron Accumulation category (Levi and Finazzi, 2014). C19orf12 encodes for a small protein found in mitochondria, endoplasmic reticulum, and at mitochondria-associated membranes. The available data suggest an involvement of the protein in lipid metabolism, mitochondrial function, and autophagy (Harting et al., 2011, Venco et al., 2015). Since in vivo models represent unique tools to study the pathomechanism of MPAN, we generated zebrafish models by a genome editing approach. The zebrafish genome contains four co-orthologues of the human C19orf12 gene: c19orf12a on chromosome 18 and c19orf12b1, b2 and b3 clustered in tandem on chromosome 7. c19orf12a protein product shares 59.6% of identity with the human protein and according to available RNA-seq data is expressed at higher levels during the early stages of zebrafish development (Mignani et al., 2020).

**Results:** A c19orf12a loss-of-function model was obtained using the CRISPR/Cas9 approach. We selected and characterized two mutant lines carrying either a 2 bp deletion (D2), inducing a premature stop codon, and an in-frame, potentially pathogenic, 3 bp deletion (D3). We performed a thorough phenotypic evaluation and an assessment of neuronal development and behavior. The spatial and temporal expression of neuronal markers by whole-mount in-situ hybridization didn’t show significant difference in the expression pattern compared to the wild-type embryos. The locomotor behavior analysis showed normal spontaneous head-tail coil movement at 24 hpf and an increase in the distance moved at 120 hpf in mutant versus wild-type siblings. Surprisingly, the D3 F3 generation was characterized by significant embryonic lethality.

**Conclusions:** These findings suggest the necessity to further investigate c19orf12 paralogues contribution in zebrafish development. Hence, we planned to perform a large-scale deletion using the CRISPR/Cas9 to remove all c19orf12 co-orthologues.
**P23. Live imaging of cell motility and mitochondrial dynamics provides a new energy-consuming mechanism required for NGF-induced differentiation**

Goglia I.1,2, Gioia C.1,3, Martorana F.1, Colangelo A.M.1

1Laboratory of Neuroscience “R. Levi-Montalcini”, Dept. of Biotechnology and Biosciences, University of Milano-Bicocca, Milano, Italy; 2PhD Program in Neuroscience, School of Medicine and Surgery, University of Milano-Bicocca, Milano, Italy; 3PhD Course in Translational and molecular medicine (DIMET), University of Milano-Bicocca, Milano, Italy

**Introduction and objectives:** Nerve Growth Factor (NGF) is a neurotrophic factor crucial for differentiation and maintenance of specific neuronal populations (Levi-Montalcini, 1987). We published that NGF-induced differentiation involves active mitochondrial remodelling, spreading them along the neurites, as well controlling of energy and redox homeostasis in response to the higher energy demand, implying a link between energy metabolism, mitophagy and mitochondrial biogenesis and dynamics (Martorana et al., 2018). NAD+ is also known to boost energy metabolism and mitophagy thus playing a critical role in neuroprotection against several toxic stimuli. NAD+ seems also to be involved in control of mitochondrial dynamics through the activation of NAD+-dependent enzymes (Massudi et al., 2012).

We aimed to compare the effect of NGF and NAD+ supplementation on neuronal differentiation and mitochondrial dynamics in PC12 cell. To this end, we used bright-field and fluorescence live imaging of cells and of mitochondrial morphology and mitophagy.

**Results:** Interestingly, we found that NAD+ alone is able to induce PC12 differentiation to an extent comparable to NGF, and potentiates NGF-induced neurites sprouting by increasing mitochondrial number, volume and mitophagy, as determined by increased colocalization of mitochondria with lysosomes. Nonetheless, NAD+ effect in neuronal differentiation is not persistent and does not produce a post-mitotic state. Moreover, we show that NGF-induced differentiation is associated with an increased cellular motility, as compared to both CTR and NAD+-dependent differentiation. The velocity and distance covered by cells peaked at 24h, suggesting an important role performed by cell motility in differentiation.

**Conclusions:** NAD+ is able to induce induces fast and transient neuronal differentiation in PC12 cells, but is not sufficient to ensure a stable neuronal differentiation. Furthermore, both NGF and NAD+ increase mitophagy and effect on mitochondrial dynamics, but with a different timing, peculiar of their different mode of action.

**Bibliography:**


P24. Development of Neural Stem Cell-based experimental therapy for the treatment of Amyotrophic Lateral Sclerosis


1Department of Biotechnology and Biosciences, University of Milano-Bicocca; 2Fondazione IRCCS, Casa Sollievo della Sofferenza; 3UPTA Unit, Fondazione IRCCS Casa Sollievo della Sofferenza; 4Neuroscience Institute Cavalieri Ottolenghi, Department of Neuroscience “Rita Levi Montalcini”, University of Turin.

Introduction and objectives: Preclinical studies demonstrated that neural stem cells (NSCs) can antagonize neuroinflammation and neurodegeneration in several neurological disease models (Pluchino et al., 2009; Rota Nodari et al., 2010; Ferrari et al., 2012). Human-NSCs (hNSCs) transplantation in the spinal cord of an Amyotrophic Lateral Sclerosis (ALS) rat model slowed the development of disease symptoms and ALS histopathological markers (SOD1 deposits, reactive astro/microgliosis, motor neurons degeneration) (Zalfa et al., 2019). These data led to the completion of a Phase I Clinical Trial (NCT01640067) for ALS patients demonstrating the feasibility of intraspinal hNSCs transplant (Gelati et al., 2013, Mazzini et al., 2015, 2019). The patient cohort was too small to draw final conclusions, however we observed a significant transitory decline of the ALS-FRS score progression up to 4 months after transplantation. Increase cell dosage should improve and extend the duration of the putative beneficial outcomes. The number of spinal cord injections is limited by the backbone destabilization consequent to the surgery. Therefore, we aim at evaluating the safety and efficacy of intracerebroventricular delivery of hNSCs as a novel strategy to increase cell dosage and favor a broader spread of the transplanted cells and of their secreted healing factors throughout the motor neuraxis by exploiting the liquor circulation.

Results: hNSCs (300,000 cells/mice) transplanted into the lateral ventricle (icv) of immunodeficient mice are well tolerated and not tumorigenic after 6 months, can extensively migrate and adhere to the ventricle wall occasionally migrating into the parenchyma. Preliminary data suggest also that hNSCs transplanted in SOD1G93A mice survive for at least 2 months with transient immunosuppression.

Conclusions: Future directions will be aimed at corroborating these preliminary results and evaluating safety and efficacy of increasing dosage (up to 1x10^6 cells) in nude mice and ALS mouse models.
Introduction and objectives: Inhibitory control is crucial in decision-making, allowing behavioral flexibility in ever-changing environments (Mirabella, 2014). This executive function is severely impaired in Parkinson’s disease (PD; Mirabella et al., 2012; 2013; 2017; Mancini et al., 2019; Di Caprio et al., 2020). Although it is known that the midbrain's dopamine neurons are crucial in decision making (Montague et al., 2004; Ryterska et al., 2013), the effects of dopaminergic treatment (DT) on inhibition are still unclear. According to the disease severity, we aim to uncover these effects on two domains of motor inhibition (Mirabella 2021), i.e., reactive and proactive inhibition. Thus, we compared the stop-signal task (SST) performance at different Hoehn and Yahr (H&Y) stages of PD. We recruited patients in the early (H&Y=1/1.5, n=20), intermediate (H&Y=2, n=20), and advanced (H&Y=2.5/3, n=20) stages, administering the SST both in ON and OFF medication (12 hours of washout). We also tested 20 age-matched healthy controls. Finally, we evaluated the effect of DT on both domains of motor inhibition.

Results: We found that both reactive and proactive inhibition are progressively impaired along the disease. However, the DT negatively affects reactive inhibition in the early and proactive inhibition in the intermediate PD stage. By contrast, DT does not impact motor inhibition in moderate/advanced patients.

Conclusions: In PD’s early and intermediate stages, the DT negatively impacts reactive inhibition in the early and proactive inhibition in the intermediate PD stage. By contrast, DT does not impact motor inhibition in moderate/advanced patients.

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P26. Generation and characterization of ap1s2 mutant lines in *Danio rerio*

**Massardi E.**, Facchinello N., Mignani L., Finazzi D., Monti E., Zizioli D., Borsani G.

1Division of Biology and Genetics, Department of Molecular and Translational Medicine, University of Brescia, Italy; 2Department of Molecular Medicine, University of Padua, Italy; 3Division of Biotechnology, Department of Molecular and Translational Medicine, University of Brescia, Italy

**Introduction and objectives:** Adaptor protein 1 (AP-1) promotes cargo sorting between trans-Golgi network and early or recycling endosomes and is composed of four subunits (γ1, β1 μ1 and σ1; Robinson, 2015). Although deficiency for the ubiquitous subunits γ1 and μ1A is embryonic lethal (Zizioli et al., 1999; Meyer et al., 2000; Zizioli et al., 2017), knockout mice of σ1B isoform are viable, but reveal impaired tissue functions (Glyvuk et al., 2010; Baltes et al., 2014). Mutations in the human orthologue AP1S2 cause the Pettigrew syndrome (MIM#304340), characterized by mental retardation and additional variable features. Our aim is to integrate previous knowledge obtained in cellular and animal models through the generation of zebrafish CRISPR/Cas9 knockout lines for ap1s2, which encodes the σ1B subunit of AP-1.

**Results:** CRISPR/Cas9 approach led to the generation of different alteration in ap1s2 coding sequence. We decided to initially study the ap1s2Δ5 line, characterized by a deletion of 5 nucleotides, which is predicted to be a frameshift mutation. Mendelian inheritance showed the expected ratio for +/- individuals at 5 dpf. Spatial-temporal expression using an antisense RNA probe for ap1s2 on wild-type AB embryos revealed a strong signal in the central nervous system at 24 and 48 hpf, which resulted to be weaker in 48 hpf +/- embryos. The effect of ap1s2 deficiency during neurogenesis was investigated through antisense RNA probes for neural markers (e.g. pax2a, ngn1 and nrd1), showing subtle alterations in +/- embryos compared to wild-type in the cranial ganglia and in midbrain-hindbrain-boundary region.

**Conclusions:** Our genome editing approach led to the generation of ap1s2 loss-of-function alleles, which will be instrumental for the in vivo characterization of this gene. Further investigations should include western blot and immunofluorescence analyses, histological sections of the zebrafish brain and behavioral studies to verify the presence of alterations of the locomotor system or the memory tasks.
P27. Gain-of-function due to increased opening probability by two KCNQ5 pore variants causing developmental and epileptic encephalopathy

Nappi M.1, Barrese V.1, Carotenuto L.1, Lesca G.2, Labalme A.2, Ville D.3, Smol T.4, Rama M.4, Dieux-Coeslier A.5, Rivier-Ringenbach C.6, Soldovieri M.V.7, Ambrosino P.8, Mosca I.7, Pusch M.9, Miceli F.1, Taglialetela M.1

1Department of Neuroscience, University of Naples “Federico II”, 80131 Naples, Italy; 2Department of Medical Genetics Lyon University Hospital, Claude Bernard Lyon 1 University, 69677 Lyon, France; 3Department of Pediatric Neurology, Lyon University Hospital, Claude Bernard Lyon 1 University, 69677 Lyon, France; 4CHU Lille, Institut de Généétique Médicale and Univ. Lille, EA 7364 – RADEME - Maladies RAREs du Développement embryonnaire et du MÉtabolisme, F-59000 Lille, France; 5CHU Lille, Clinique de Génétique – Guy Fontaine, F-59000 Lille, France; 6Department of Pediatrics, Hôpital Nord-Ouest, Villefranche-sur-Saône, 69400, France; 7Department of Medicine and Health Science, University of Molise, 86100 Campobasso, Italy; 8Department of Science and Technology, University of Sannio, 82100 Benevento, Italy; 9Institute of Biophysics, Italian National Research Council, 16149 Genova, Italy

Introduction and objectives: The M-current (I\textsubscript{KM}) is a potassium current that critically regulates neuronal excitability. Although heteromeric channels composed of KCNQ2 and KCNQ3 subunits are believed to provide a major contribution to I\textsubscript{KM} in adult neurons (Shah et. al., 2002), KCNQ5 subunits may contribute to IKM molecular heterogeneity (Schroeder et. Al., 2000). It was recently reported that missense mutations in KCNQ5 genes are responsible for developmental and epileptic encephalopathies (DEEs) (Lehman et. al., 2017), a neurodevelopmental diseases characterized by refractory epilepsy, distinct EEG and neuroradiological features, and various degrees of developmental delay (Scheffer et. al., 2017).

In the present work, the clinical features of two DEE patients carrying de novo KCNQ5 variants affecting the same residue in the pore of Kv7.5 subunits (G347S/A) are described. The in vitro functional properties of channels incorporating these variants were also investigated. Site-directed mutagenesis was used to introduce specific mutations in plasmids containing the cDNA for Kv7.5, Kv7.2 and Kv7.3 subunits. Electrophysiological and biochemical experiments were performed in CHO cells transiently transfected with these constructs.

Results: Currents carried by Kv7.5 G347S/A channels displayed functional features consistent with a strong gain-of-function (GoF) in vitro functional phenotype. Similar functional changes were also observed when the same variants were introduced at the corresponding position in Kv7.2 subunits. Nonstationary noise analysis revealed that GoF effects observed for both Kv7.2 and Kv7.5 variants were mainly attributable to an increase in single channel open probability, without changes in membrane abundance or single channel conductance. In addition, the mutation-induced increase in channel opening probability was insensitive to manipulation of membrane levels of the critical Kv7 channel regulator PIP\(_{2}\).

Conclusions: These results reveal a novel pathophysiological mechanism for KCNQ5-related DEEs. Functional characterizations of de novo variants are crucial to understand pathogenesis of DEEs, to guide therapy and to implement personalized treatments.
P28. AMPA and NMDA receptors expression modifications in the PFC of rat model of PTSD and after ketamine treatment

Ndoj E.¹, Mingardi J.¹,², Carini G.¹, La Via L.¹, Musazzi L.², Barbon A.¹
¹Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy; ²School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy

Introduction and objectives: Traumatic stressful experiences are well-known factors able to mediate the vulnerability to psychiatric disorders, in particular post-traumatic stress disorder (PTSD). PTSD is often set off by a single, acute traumatic event that might negatively impact on the body homeostasis and induce long-term maladaptive changes in brain functions and connectivity. The biological mechanisms underlying these effects are not fully understood yet, however different studies suggest that dysregulation of glutamatergic synaptic functions may play a key role in the pathophysiology of PTSD. Further, new insights are offered by works investigating a possible therapeutic role of the fast-acting antidepressant ketamine. A single session of foot shock (FS), an acute paradigm of stress, induced an amplification of depolarization-evoked glutamate release in the prefrontal cortex (PFC) of rats, together with remodeling of pyramidal neurons dendritic arborization (Sala et al., 2022). These changes were fully rescued in 24 h by a single sub-anesthetic dose of ketamine. Here, we aimed to observe if FS induces changes in the expression of AMPA and NMDA glutamate receptors and if ketamine acts on this class of receptors. For this reason, rats were subjected to a single session of FS and, after 6 h, stressed rats were treated with ketamine. Animals were sacrificed after 24 h and PFC homogenized (OMO) and synaptic membrane fractions (LP1) were prepared for western blot experiments.

Results: where FS induced an increase in AMPA GluA2 Ser⁸⁸⁰ expression, rescued by ketamine and a decrease in GluA1 Ser⁸⁴⁵ in the homogenate fractions. Furthermore, NMDA GluN2A expression was significantly increased in FS-stressed rats and ketamine reversed the alteration.

Conclusions: The changes in the NMDA/AMPA receptors expression found in rats subjected to FS and acute ketamine might corroborate the involvement of the glutamatergic system in the stress response together with suggesting the ability of ketamine to act at this level.
P29. Repurposing pomalidomide as a neuroprotective drug in an alpha-synuclein-based model of Parkinson’s disease


1Department of Biomedical Sciences, University of Cagliari, Italy; 2Department of Biomedical and Biotechnological Sciences, University of Catania, Italy; 3CNR Institute of Translational Pharmacology, Cagliari, Italy; 4Department of Pharmacy, University of Naples “Federico II”, Italy; 5Centre for Misfolding Diseases, Department of Chemistry, University of Cambridge, Cambridge, UK; 6Department of Life and Environmental Sciences, University of Cagliari, Italy; 7Drug Design & Development Section, Translational Gerontology Branch, Intramural Research Program, National Institute on Aging, National Institutes of Health, Baltimore, MD, United States; 8National Research Council, Institute of Neuroscience, Cagliari, Italy

Introduction and objectives: The development of therapeutic approaches to slow or even stop disease progression remains the greatest unmet therapeutic need in Parkinson’s disease (PD). However, given the high cost and low success rate in new drug development, a complementary strategy based on repositioning drugs that are approved for other indications should be taken into consideration (Jung et al., 2021). Here, we tested for the first time the disease-modifying properties of the immunomodulatory imide drug (IMiD) pomalidomide in a translational rat model of PD based on the intranigral bilateral infusion of toxic oligomers of human α-synuclein (H-αSynOs). The neuroprotective effect of pomalidomide (20 mg/kg; i.p. three times/week) was tested in the first stage of disease progression by means of a chronic two-months administration.

Results: The infusion of H-αSynOs induced an impairment in motor performance that was fully rescued by pomalidomide, as assessed via a battery of motor tests. Moreover, H-αSynOs-infused rats displayed a 40–45% cell loss within the substantia nigra (SN), as measured by stereological counting of TH⁺ and Nissl-stained neurons, that was largely abolished by pomalidomide. The inflammatory response to H-αSynOs infusion and the pomalidomide treatment was evaluated both in CNS and peripherally. A reactive microgliosis was present in the SN three months after H-αSynOs infusion as well as after H-αSynOs plus pomalidomide treatment. However, microglia differed for their phenotype among experimental groups. After H-αSynOs infusion, microglia displayed a proinflammatory profile, producing a large amount the cytokine TNF-α. In contrast, pomalidomide inhibited the TNF-α overproduction and elevated the anti-inflammatory cytokine IL-10. Moreover, the H-αSynOs infusion induced a systemic inflammation with overproduction of serum proinflammatory cytokines and chemokines, that was largely mitigated by pomalidomide.

Conclusions: Finally, we provide evidence of the disease modifying potential of pomalidomide in a progressive model of PD, thus adding a possible rationale for clinical testing of this drug in PD patients.

Bibliography:

Niemann-Pick type C1 disease (NPC) is a fatal and rare autosomal recessive lysosomal disorder resulting from mutation in NPC1 gene. It is caused by an abnormal lipid transport and accumulation of unesterified cholesterol in endosome-lysosome system. Patients show different visceral and neuropsychiatric symptoms that induce a severe cognitive impairment (Pallottini and Pfrieger, 2020) and to date there are no curative therapy. Most of the NPC1 mutations produce proteins which are immediately degraded but presenting residual activity (Ebrahimi-Fakhari, 2016). Therefore, increasing the availability of mutant NPC1 protein with residual activity in the correct subcellular compartment could be an emerging therapeutic approach to restore the physiologic turnover of cholesterol in the cells. We found out, in a hepatic-derived cell line, that an epigenetic pathway regulated by BET (Bromodomain and Extra-Terminal motif) proteins – amenable to pharmacologic manipulations – controls NPC1 protein expression, together with other proteins involved in cholesterol homeostasis (Tonini et al., 2020). Thus, the aim of our work is to investigate whether the modulation of BET proteins can increase NPC1 protein level and reduce cholesterol accumulation in both human-derived cells carrying NPC1 mutations and transgenic mouse. Therefore, fibroblasts derived from NPC1 patients have been treated with a specific BET protein inhibitor (JQ1).

Our results show that, in some mutant cells, JQ1 strongly increases NPC1 protein expression, reduces cholesterol accumulation, and rescues some proteins involved in cholesterol metabolism. Altogether, our data suggest that BET proteins are regulators of cholesterol metabolism and since this epigenetic pathway is amenable to pharmacologic manipulation, it could be a good drug target for NPC1 disease.

Bibliography:


Introduction and objectives: Cannabidiol (CBD) is the second most abundant phytocannabinoid in Cannabis sativa after trans-Δ9-tetrahydrocannabinol, and it is devoid of euphoric action. Recently, several studies have concluded that CBD is a promising therapy in autism spectrum disorders (ASD) to support forthcoming clinical trials (Aran et al., 2019; 2020; Poleg et al., 2018). However, the potential efficacy and mechanisms of CBD in many neuropsychiatric disorders and in particular in ASD have not yet been well explored. In order to define the molecular mechanisms of CBD, in vitro tests have been performed. In particular, we investigated the crosstalk between oxytocin and endocannabinoids. Furthermore, CBD effect on social behavior has been tested in vivo.

Results: In vitro experiments on two different cell lines have demonstrated that CBD treatment induces an increase in oxytocin expression levels and this increase is mediated by the presence of the TRPV2 receptor. Finally, a sub-chronic treatment (14 days) with CBD oil increased social interactions in wild type mice, and in this action, the oxytocin (OXY) pathway is involved.

Conclusions: These data show that CBD treatment is able to increase social behavior in mice. This effect is mediated by oxytocin and the data obtained in vitro support the involvement of the TRPV2 receptor. Due to both the social behavior and the oxytocin pathway alterations present in autism spectrum disorders patients, the use of CBD could represent a new interesting therapeutic strategy.
P32. New antioxidant K+ channel-independent effect of XE-991 in an in vitro model of metabolic impairment: implications for Alzheimer’s Disease

Preziuso A.1, Piccirillo S.1, Amoroso S.1, Serfilippi T.1, Miceli F.2, Magi S.1, Lariccia V.1.

1Department of Biomedical Sciences and Public Health, School of Medicine, University “Politecnica delle Marche”, Via Tronto 10/A, 60126, Ancona, Italy; 2Department of Neuroscience, University of Naples, “Federico II”, Via Pansini 5, 80131, Naples, Italy.

Introduction and objectives: Alzheimer’s disease (AD) is a progressive neurodegenerative disorder representing the leading cause of dementia. It is well known that oxidative stress, metabolic alterations, and mitochondrial dysfunction play an important role in the progression of AD (Moreira et al., 2010). As a result, over the past years, remarkable efforts have been made to develop neuroprotective strategies against oxidative damage and mitochondrial impairment (Misrani et al., 2021). This work aimed at evaluating the antioxidant property of the inhibitor of the M-current (IKM) XE-991, since it is known that some IKM enhancers exert a neuroprotective effect by counteracting oxidative stress (Seyfried et al., 2000).

Results: In the present study, we discovered a novel antioxidant K+ channel-independent effect of the IKM inhibitor XE-991 in retinoic acid differentiated SH-SY5Y cells and rat primary cortical neurons exposed to the glycolysis inhibitor glyceraldehyde (GA). This compound was used to induce a condition of hypometabolism accompanied by mitochondrial dysfunction and redox imbalance, thus reproducing the early stage of AD (Magi et al., 2021). We found that XE-991 exerted a neuroprotective action most likely through the resumption of superoxide dismutase (SOD) activity, which was significantly compromised during GA challenge. We also observed that the increase of SOD activity occurred in parallel with the reduction in both cytoplasmic and mitochondrial Ca2+ levels, the decrease in mitochondrial reactive oxygen species production, the modulation of AMPK/mTOR pathway, the recovery of mitochondrial membrane potential collapse, the increase in the intracellular ATP content and the decrease in Aβ and pTau levels.

Conclusions: This study demonstrated that XE-991 could exert an antioxidant effect based on a K+ channel-independent mechanism, which possibly involves the enhancement of SOD activity. Our findings may pave the way toward the further evaluation of already existing molecules, to accelerate the development of an effective therapy to counteract AD.
Nowadays, our organism is exposed to a variety of stressors (due to exhausting lifestyle, increased population density, pollutants and environmental/global changes), responsible of the accumulation of cellular alterations predisposing to the pathology. This could justify the increasing incidence of neurodegenerative diseases, as Amyotrophic Lateral Sclerosis (ALS) (Bongioanni et al., 2021). ALS is a motor neuron (MN) disease, determining weakness, muscle atrophy and premature death: it is characterized by excitotoxicity, oxidative stress and neuroinflammation, cellular processes similarly triggered by stress (Peña-Bautista et al., 2020).

This work aims to clarify the contribution of different stressors in causing/anticipating ALS, since many mechanisms are still unknown. Preliminary experiments have been set-up in vitro, using NSC-34 cells expressing hSOD1(G93A) gene under the control of a doxycycline-inducible promoter. To differentiate the NSC-34 cells in MN-like cells, different retinoic acid (RA) concentrations have been tested: RA (1, 5, 10, 15 or 20μM) was added to the culture medium for 2, 4, 6 and 8 days. Based on the MTT assay results, 20μM RA for 4 days represents the most proper condition to induce cell maturation. Concerning the overexpression of hSOD1(G93A), the cells were grown in complete medium and 5μg/ml of doxycycline for 24h: hSOD1 expression was confirmed by WB, both in undifferentiated and differentiated cells. Finally, to mimic a stress condition, cells underwent oxygen glucose deprivation: CoCl2 was used as hypoxic agent and its toxicity was measured by MTT assay: 50, 100, 200, 300 or 400μM of CoCl2 were evaluated in both high and low glucose medium. Preliminary results suggest that 100μM CoCl2 in low glucose represents the optimal stress condition. With these preliminary experiments, we have set-up the conditions for the following analyses, to evaluate genetic/epigenetic mutations and cellular/molecular alterations, and to clarify the stressor impact on the CNS and on the predisposition to neurological pathologies.
Autosomal Dominant Leukodystrophy (ADLD) is a rare genetic disease, characterized by autonomic dysfunction and movement disorder, and associated with white matter loss in the central nervous system (CNS). The genetic cause is the presence of three copies, instead of the two normally present, of the gene that contains the instructions to produce the lamin B1 (LMNB1) protein, which belongs to a group of structural proteins forming the nuclear membrane of the cell. Although it is well known that LMNB1 regulates nuclear mechanics and integrity, interacts with chromatin, and regulates gene expression, pathogenic mechanisms in ADLD have only initially been explored. Moreover, a therapy to treat this disease is not available at the moment. Disease-relevant human models are therefore crucial to study disease pathogenesis and to further screen for effective therapies.

Based on evidence showing glial pathology in ADLD patients, we set out to generate human glia including both oligodendrocytes and astrocytes from healthy donors (CTRL) and ADLD human induced pluripotent stem cells (hiPSCs). Toward this goal, we established a differentiation protocol based on three stages: the commitment to neural progenitors, the production of gliospheres and a further maturation step into authentic oligodendrocytes and astrocytes. Preliminary observations indicate a lower gliogenic potential in the hiPSC ADLD lineages, as revealed by gliosphere production. In ADLD astrocytes, an altered expression of LMNB1 is confirmed by RT-qPCR and protein expression, together with morphological alteration affecting the nuclear shape that in turn impacts certain morphological features of the cell body (ramifications, area covered).

Functional analyses will be devoted to investigating possible alterations correlated with the LMNB1 higher expression. Thus, the developed model appears as a promising "disease-in-a-dish" platform to further reveal so far unknown dysfunctions of the diseased cells and, prospectively, aid the development of effective therapeutic strategies for this rare genetic disease.
Introduction and objectives: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects motor neurons (MNs), leading to their death. Only symptomatic treatments are available and, for this reason, it is important to find a realistic model to study its aetiopathogenesis (Pansarasa et al., 2022). Cerebral organoids are pluripotent stem cells-derived self-organizing structures, which allow the study of brain development (Bordoni et al., 2018). Aim of this work was the development of a protocol for motor neuron organoids (MNOs) generation and their characterization.

To reach this aim, we differentiated induced pluripotent stem cells from healthy control and sALS patients into neural stem cells (NSCs). NSCs were differentiated first into MNs progenitors and finally in mature MNs for 2D cultures. For the generation of MNOs, NSCs were dissociated and cultured in floating conditions and then differentiated. We characterized MNOs by phase-contrast and confocal microscopy and we performed RNAseq to compare 2D MNs cultures and MNOs. Finally, we investigated DNA methylation by enzyme-linked immunosorbent assay (ELISA).

Results: sALS MNOs were smaller, with an irregular morphology and a thicker glial layer compared to healthy MNOs. Moreover, healthy control MNOs showed longer neurites compared to sALS MNOs and a higher amount of differentiated cells. RNAseq showed a ten-fold increase of deregulated genes in MNOs respect to 2D cell models. Furthermore, sALS MNOs exhibited a considerable deregulation of genes involved in axonogenesis, axon guidance and extracellular matrix organization. Finally, ELISA showed a significant reduction of methylation status of sALS MNOs when compared to 2D sALS MNs.

Conclusions: these data confirm that brain organoids can be considered a promising model for the study of ALS. We found the typical hallmarks of ALS pathology, such as the decreased levels of MNs, which showed a reduction in neuritis length, and a deregulation of genes involved in the disease.
P36. Morpho-functional alterations in epithelial cells of the Choroid Plexus during aging

Scarpetta V.1,4, Bodaleo Torres F.2, Salio C.3, Sassoè-Poggetto M.1, Agarwal A.2, Patrizi A.4
1Department of Neurosciences, University of Turin, Turin, Italy; 2Chica and Heinz Schaller Research Group, Institute for Anatomy and Cell Biology, Heidelberg, Germany; 3Department of Veterinary Sciences, University of Turin, Turin, Italy; 4Schaller Research Group, German Cancer Research Center (DKFZ), Heidelberg, Germany

The choroid plexus (ChP), a specialized vascular-epithelial structure located in the brain ventricular system, makes a selective interface between blood and cerebrospinal fluid (CSF), thus regulating brain homeostasis (Ghersi-Egea et al., 2018). It has been postulated that age-associated neurodegenerative diseases can be correlated with alterations of ChP function (Balusu et al., 2016). Here we examined the ultrastructure of the ChP in adult (P60) and aging (P600) mice using electron microscopy. We identified different aging-related changes, including a reduction of epithelial cell height, a decrease in the area covered by apical microvilli, and a structural modification of tight junctions. We also found a redistribution of mitochondria along the basal-apical cell axis and a significant increase in the density of elongated mitochondria in old mouse ChP. Interestingly, 2-photon (2PM) imaging of whole ChP living explants and mitochondria tracking analysis revealed that mitochondria mainly show a wiggling movement, a feature that was rather constant throughout lifetime. Our data demonstrate that the choroid epithelium undergoes multiple structural alterations during aging. In particular, changes in the shape of mitochondria could correlate with increased vulnerability to metabolic stress (Gouras et al., 2016; Li et al., 2017).
**P37. Inflammatory pathway dysregulations promote amyloid beta precursor protein phosphorylation on Tyr682 residue in monocytes from patients with Alzheimer’s disease**

Serafini S.\(^1\), Ferretti G.\(^1\), Angiolillo A.\(^2\), Di Costanzo A.2, Frisardi V.\(^3\), Maier J T.\(^4\), and Matrone C.\(^1\)

\(^1\)Unit of Pharmacology, Department of Neuroscience, Faculty of Medicine, University of Naples Federico II, Naples, Italy; \(^2\)Center for Research and Training in Medicine of Aging, Department of Medicine and Health Sciences, University of Molise, Via F. De Sanctis 1, 86100, Campobasso, Italy; \(^3\)Paul-Ehrlich Institute, Federal Institute for Vaccines and Biomedicines, Langen, Germany

**Introduction and objectives:** Alzheimer’s Disease (AD) is a neurodegenerative disease characterized by cognitive deficits and microscopic brain changes such as A\(\beta\) plaques and twisted strands of tau protein. These changes are associated to neuronal death, atrophy or shrinkage of specific brain areas and extensive neuroinflammation (Abubakar et al., 2022). Our group has previously emphasized the role of APP Tyr\(_{682}\) residue as novel druggable signature in AD (Matrone et al., 2019). APP Tyr\(_{682}\) residue is located in the highly conserved \(682^{\text{YENPTY}}_{687}\) motif, which binds specific adaptor proteins depending on its phosphorylation state (Guénette et al., 2017). The increased APP Tyr\(_{682}\) phosphorylation affects APP endocytosis and trafficking inside neurons and causes APP accumulation to generate A\(\beta\) peptides (Iannuzzi et al., 2020). Fyn tyrosine kinase (TK) elicits APP Tyr682 phosphorylation and triggers amyloidogenic processes in AD neurons (Poulsen et al., 2017). APP Tyr\(_{682}\) phosphorylation increases in neurons (Iannuzzi et al., 2020), fibroblasts (Iannuzzi et al., 2021) and blood monocytes from AD patients (Reveglia et al., 2021). We here questioned whether peripheral blood samples from patients showing clinical and/or neuroimaging signs of AD or mild cognitive impairment (MCI) might be neurotoxic for human healthy microglia.

**Results:** We exposed human microglia to plasma from 10 AD, 10 MCI and 10 healthy volunteers for 8hrs. We found that plasma from most of the MCI and AD patients activated an inflammatory phenotype in microglia and increased APP Tyr\(_{682}\) phosphorylation and A\(\beta\) peptide production. Notably, microglia did not die when exposed to plasma from affected patients but rather showed a dysregulated proliferation of microglia progenitors (small round cells). Differently, plasma from healthy volunteers did not affect microglia proliferation nor APP Tyr\(_{682}\) phosphorylation and processing.

**Conclusions:** These findings underline a new potential neurotoxic mechanism in which peripheral inflammatory factors might activate A\(\beta\) pathology in microglia and initiate neurodegeneration in brain.
Introduction and objectives: Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by Amyloid β(Aβ)-driven synaptic dysfunction in the early phases of pathogenesis. In addition to spine loss, cytoskeletal abnormalities, such as cofilin-actin rods, have been reported in AD patients and animal models (Bamburg et al., 2010). Cytoplasmic rods contain cofilin and actin and are formed upon exposure to different stressors, including Aβ oligomers (Bamburg at al., 2016). We have recently demonstrated that the actin-binding protein Cyclase-associated protein 2 (CAP2) is a master regulator of cofilin localization and activity, through the Cys32-dependent CAP2 dimerization. These mechanisms are altered in AD, suggesting an involvement of CAP2/cofilin pathway in AD pathogenesis (Pelucchi and Vandermeulen et al., 2020). This work aims to explore the involvement of CAP2 in Aβ-induced actin rods formation during the early phases of AD pathogenesis.

Results: Firstly, we demonstrated that Aβ oligomers impair CAP2/cofilin pathway both after a short exposure and when cofilin-actin rods are generated, although the resulting effects are different. Then, we found that CAP2 accumulates in actin rods, when specifically induced by Aβ exposure, but not when neurons are exposed to a different stressor. Finally, we show that CAP2 overexpression can prevent rods formation and spine loss, through a mechanism that requires CAP2 capability to form Cys32-dependent dimers.

Conclusions: Overall, our data support the involvement of cofilin/CAP2 cooperation in different biological aspects of AD pathogenesis in neuronal cells, thus providing novel potential therapeutic target for AD.

Bibliography:


P39. The negative allosteric modulator CTEP ameliorates the reactive phenotype of i-astrocytes from patients affected by Amyotrophic Lateral Sclerosis

Tessitore S.¹, Torazza C.¹, Kumar M.¹, Allan S.², Shaw P. J.², Ferraiuolo L.², Bonanno G.¹,³, Milanese M.¹
¹University of Genoa, Department of Pharmacy-Pharmacology and Toxicology Unit, Genoa, Italy; ²University of Sheffield, Sheffield Institute of Translational Neuroscience (SiTrAaN), Sheffield, United Kingdom; ³IRCCS, Ospedale Policlinico San Martino, Genoa, Italy.

Introduction and objectives: Amyotrophic Lateral Sclerosis (ALS) is a non-cell-autonomous neurodegenerative disease. Glutamate-mediated excitotoxicity is a major cause of motor neuron degeneration, and the metabotropic type 5 glutamate receptor (mGluR5) plays a key role in shaping glutamate effects. Here we investigated the impact of in-vitro mGluR5 inhibition by the selective negative allosteric modulator 2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazol-4-yl)ethynyl)pyridine (CTEP) on the phenotype of inducible neural progenitor cell (iNPC)-derived astrocytes (i-astrocytes) from ALS patients and healthy donors.

Results: Western blot and immunofluorescence showed reduced expression of the reactive astrocyte markers GFAP, S100β, and C3 in C9orf72 and SOD¹⁴⁴V iNPC-derived i-astrocytes following x day-exposure in culture to 10nM CTEP vs. healthy donor controls. The pharmacological inhibition with CTEP did not up- or down-regulated the mGluR5 expression. CTEP also reduced the expression of NLRP3 and increased the expression of Nrf2, markers of inflammation and oxidative stress response, respectively. Next, we tested the activity of four enzymes linked to antioxidant cellular defenses (glutathione reductase, glutathione peroxidase, glucose-6-phosphate dehydrogenase, catalase), which we found increased in C9orf72 and SOD¹⁴⁴V iNPC-derived astrocytes. CTEP reduced the activity of the four enzymes, suggesting an improvement in the redox state. Consequently, CTEP-treated C9orf72 and SOD¹⁴⁴V iNPC-derived astrocytes showed reduced malondialdehyde expression as a marker of lipid peroxidation. CTEP did not modify the above mentioned parameters in healthy donors.

Conclusions: Modulation of mGluR5 by CTEP treatment positively impacted on the reactive phenotype of i-astrocytes of patients with C9orf72 and SOD¹⁴⁴V ALS, extending our previous in-vitro and in-vivo results with the SOD¹⁴⁴⁹⁹A mouse model of human ALS.
P40. Induced pluripotent stem cell-derived neurons as a model to study neuronal alterations and stress responses in patients with Treatment-Resistant Depression

Tomasoni Z.1, Bono F.1, Mingardi J.2, Sbrini G.1, Minelli A.2,3, Guglielmi A.1, Gennarelli M.2,3, Missale C.1, Barbon A.2, Fiorentini C.1

1 Division of Pharmacology, Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy; 2 Division of Biology and Genetics, Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy; 3 Genetic Unit, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy

Introduction and objectives: Although major depressive disorder (MDD) is a leading contributor to global burden of disease (Ferrari et al., 2013), the underlying pathophysiology is still poorly understood. Moreover, about 30% of patients with MDD do not respond to traditional treatments, and they are classified as having treatment-resistant depression (TRD) (Al-Harby, 2012). A key role in the pathogenesis of MDD has been attributed to the interaction between genetic background and environment; in particular, stress is the major environmental risk factor for the development of MDD (Keers and Uher, 2012). Several studies on stress-based animal models of depression and MDD patients have demonstrated volumetric changes in cortico-limbic brain areas and alterations in neuronal morphology (Duman et al., 2019); moreover, antidepressant treatment ability in reversing morphological neuronal defects has been described (Bessa et al., 2009).

Therefore, in this study we analyzed the cellular and morphological characteristics of glutamatergic, dopaminergic and GABAergic neurons derived from induced pluripotent stem cells (iPSCs) from two TRD patients (Bono et al., 2020, 2021), both at basal condition as well as following cortisol treatment, as a methodological strategy to mimic a stress event. Number and morphological characteristics of iPSCs-derived neurons were analyzed using a combination of semi-quantitative PCR and immunofluorescence for the analysis of neuronal markers of differentiation and maturation and measurement of dendritic branches and soma area.

Results: Among our results, quantitative analyses of the different neuronal populations at the end of differentiation protocol have shown alterations in the number of GABAergic and glutamatergic neurons in both TRD patients, that is in line with the imbalance between inhibitory and excitatory pathways, consistently associated with MDD (Lener et al., 2017).

Conclusions: Therefore, the iPSC technology could be a useful approach for identifying specific molecular abnormalities for each patient likely contributing to the complex mechanisms that lead to resistance to antidepressant drugs.
P41. Investigating the molecular pathways leading to neuroinflammation in A53T-α-Synuclein Transgenic Old Mice

Tufano M., Sisalli M. J., Sirabella R., Scorziello A.

Division of Pharmacology, Department of Neuroscience, School of Medicine, Federico II University of Naples, Via S. Pansini 5, 80131 Napoli, Italy

Parkinson’s disease (PD) is the second most common neurodegenerative disease with an high socioeconomic impact for health system, characterized by nigrostriatal degeneration. The clinical spectrum includes motor and non-motor features in the late and early stage of the disease respectively. The loss of the dopaminergic neurons is caused by the accumulation of α-synuclein in Lewy bodies and neuritis as well as mitochondrial and lysosomal dysfunction. Particularly it has been demonstrated that dopaminergic neurons exhibit a pacemaker activity which is responsible for the generation of action potentials even in the absence of synaptic inputs. In this condition, the neurons are exposed to large Ca^{2+} transients, overstimulating ion channels and transporters to prevent the increase of Ca^{2+} and neuronal death. Recently, we demonstrated an alteration of intracellular Ca^{2+} homeostasis due to a decrease of Na^{+}-Ca^{2+} exchanger isoform 3 (NCX3) expression and activity in midbrain neurons obtained from mice carrying the human mutation of α-synuclein A53T.

We investigated the molecular pathway correlating mitochondrial dysfunction to neuroinflammatory process in PD. We performed in vitro and in vivo experiments in mice A53T. The expression and activity of NCX, were evaluated in neurons and glial cells. Mitochondrial function was monitored with confocal microscopy and fluorescent dyes to measure mitochondrial Ca^{2+} content and membrane potential in striatal and mesencephalic neurons/astrocytes. In vivo experiments were performed in 4-16-month-old transgenic mice in the two above mentioned brain areas to explore mitochondrial dysfunction and neuroinflammation with biochemical analysis.

We found: 1. in A53T mice mitochondrial dysfunction occurs early in midbrain and later in striatum; 2. mitochondrial dysfunction, occurring in the midbrain, is mediated by the impairment of NCX3 protein in neurons and astrocytes; 3. mitochondrial dysfunction, occurring early in midbrain, triggers neuroinflammation later into the striatum, contributing to PD progression during mice aging. Further experiments are in progress in order to clarify the nigrostriatal pathways mediating these effects.
P42. Generation of a novel human alpha-synuclein transgenic zebrafish line as a model to study the biological basis of human synucleinopathies

Zini S. *1, Muscò A. *1, Longhena F.1, Borsani G.2, Zizioli D.#3, Bellucci A. #1

1Section of Pharmacology, Department of Molecular and Translational Medicine, University of Brescia, Viale Europa, 11, 25123, Brescia (Italy); 2Section of Biology and Genetics, Department of Molecular and Translational Medicine, University of Brescia, Viale Europa, 11, 25123, Brescia (Italy); 3Section of Biotechnologies, Department of Molecular and Translational Medicine, University of Brescia, Viale Europa, 11, 25123, Brescia (Italy)

Introduction and objectives: The deposition of insoluble alpha-synuclein (αSyn) aggregates is believed to play a primary role in neuronal loss in Parkinson’s disease (PD), Lewy body dementia (LBD) and multiple system atrophy (MSA), neurological disorders defined as synucleinopathies (Bellucci et al., 2016; 2017). Although a plethora of experimental models reproducing the different features of human synucleinopathies have been developed (Koprich et al, 2017; Outeiro et al., 2021), we still have poor knowledge on the physiological functions of αSyn and on the pathological role of its aggregates. Interestingly, zebrafish transient transgenic models have recently emerged as useful tools to study αSyn (Weston et al, 2021).

The aim of this study was to develop a novel stable human αSyn transgenic zebrafish line to investigate αSyn physiological and pathological role in neuronal cells.

Results: We generated a zebrafish transgenic line that ubiquitously expresses N-terminally-tagged mCherry human αSyn in the nervous system under the guidance of zebrafish HuC promoter by exploiting the Tol2 transgenesis system (Tg(pTol2HuC:mCherry/hSNCA)), by microinjecting one-cell-stage embryos. From 10 hours post fertilization (hpf) until 120 hpf, the Tg(pTol2HuC:mCherry/hSNCA) embryos did not exhibit significant morphological changes, abnormal cell death or behavioral abnormalities. The adult Tg(pTol2HuC:mCherry/hSNCA) F0 founders reached sexual maturity and their progeny (F1) expressed mCherry fluorescence from early developmental stages in central and peripheral neurons. Human αSyn expression was verified on both F0 and F1 embryos through reverse transcription PCR and western blot analysis.

Conclusions: The results support that the Tg(pTol2HuC:mCherry/hSNCA) zebrafish may represent a new promising model for studying αSyn-related diseases and investigate possible therapeutic targets.
P43. Social buffering enhances extinction of traumatic memory in a chronic PTSD-like model in rats
Blasi E., Campolongo P., Morena M.
Department of Physiology and Pharmacology, University of Rome “La Sapienza”, Rome, Italy; European Center for Brain Research (CERC), Santa Lucia Foundation, Rome, Italy

Introduction and objectives: Social buffering is a phenomenon by which affiliative social partners mitigate the response to stressors (Kiyokawa et al., 2017). Numerous studies reported that this phenomenon can occur equally among familiar and unfamiliar conspecifics in a variety of species, including laboratory rats (Mikami et al., 2020; Hennessy et al., 2009). Recent studies have reported that social buffering facilitates extinction of aversive memories (Mikami et al., 2020; Mikami et al., 2016).

Based on this evidence, the aim of the present study was to evaluate the efficacy of social buffering in the facilitation of traumatic memory extinction in a chronic rat PTSD-like model recently developed in our laboratory (Berardi et al., 2016; Colucci et al., 2020).

Specifically, after one week of social isolation and a footshock trauma, rats were exposed to several spaced extinction sessions to mimic the human cognitive behavioral therapy (Colucci et al., 2020). To evaluate the influence of social buffering, extinction sessions were carried out in the presence (or absence) of a social conspecific partner.

Results: Our results show that social interaction reduced fear responses (i.e., freezing behaviour) during exposure to the extinction sessions as compared to rats tested in the absence of a conspecific, thus showing the efficacy of social buffering in promoting extinction of traumatic memory in a rat model mimicking PTSD-like symptomatology.

Conclusions: Taken together, our findings provide the basis for more mechanistic studies aimed at understanding the neural underpinnings of social buffering of fear and highlight the beneficial effects of group therapy for the treatment of trauma-related disorders, such as PTSD.
P44. Cannabidiol rescues autistic-like traits in a genetic model of autism based on FMR1 deletion in rats

Buzzelli V.¹, Manduca A.¹, Carbone E.¹, Rava A.¹, Schiavi S.¹, Micale V.², Kuchar M.³, Trezza V.¹
¹Dept. of Science, University “Roma Tre”, Rome, Italy; ²Dept. Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy; ³Dept. Chemistry of Natural Compounds, University of Chemistry and Technologies, Prague, Czech Republic

Introduction and objectives: Fragile X syndrome (FXS) is the most common known inherited single-gene causes of autism spectrum disorder (ASD), with no specific treatment available (Maurin T. et al., 2014). Recently, the use of cannabinoid compounds, especially cannabidiol (CBD), an abundant bioactive but non-psychotomimetic constituent of Cannabis sativa, has received increasing attention as treatment for the core symptoms and co-morbidities of ASD. Beyond anecdotal reports, however, there is limited current evidence supporting such an intervention and the use of CBD remains controversial. In the present work, we tested the effect of CBD in the autistic-like features displayed by the recently validated Fmr1-Δexon 8 rat model of ASD. Wild-type (WT) and Fmr1-Δexon 8 male rats on a Sprague-Dawley background were used. We assessed the behavioral effects of systemic administration of CBD in these animals through the novel object recognition, three-chamber and social discrimination tests from adolescence to adulthood and explored the underlying mechanisms.

Results: Our results showed that CBD rescued the cognitive and social dysfunctions displayed by Fmr1-Δexon 8 animals. Interestingly, intra-hippocampal blockade of the GPR55 receptors prevented the ability of systemic CBD to normalize the cognitive performance of Fmr1-Δexon 8 rats, thus suggesting that the effects of CBD in Fmr1-Δexon 8 rats are mediated, at least in part, by antagonism of the lipid-activated G protein-coupled receptor GPR55 in the hippocampus.

Conclusions: These findings demonstrated that CBD reduced autistic-like traits in a genetic model of autism based on FMR1 deletion in rats and provide initial mechanistic insights into its beneficial actions in ASD.
Introduction and objectives: Notoriously, Multiple Sclerosis (MS) can impair several cognitive domains, including sustained attention, information processing speed, memory, and executive functions (Amato et al., 2006). MS patients may also experience language difficulties in everyday life (i.e., verbal fluency, lexical access, language comprehension, pragmatics) (Renauld et al., 2016; Carotenuto et al., 2018). Language difficulties have been described frequently associated with other cognitive deficits (Lebkuecher et al., 2021). Based on these findings, the present study aims to investigate the patients’ self-perceived language difficulty, assessed by the SMAC questionnaire (Riccardi et al., 2021), and its relationships with the neuropsychological performance, in particular with WLG. Neuropsychological evaluation was performed by using the Brief Repeatable Battery of Neuropsychological Tests (BRB-NT) (Rao, 1990); patients who had at least 2 tests with z score below 2 standard deviations were considered cognitively impaired (CI). Pearson correlation and regression analysis were used to explore the study’s hypothesis, also examining demographic and clinical data.

Results: 169 MS patients (female 136; 80.5%; mean age 45.41 ± 11.65 ys, education 12.67 ± 3.5 ys) were included, of these 78 (46.2%) presented with cognitive impairment (CI). MS duration and EDSS were 12.89 ± 9.6 and 2.53 ± 1.72 ys. Independent T-test shows a significant difference of WLG z between CI and not CI patients (p<0.001). A correlation between WLG and SMAC (p=0.024) was reported by Pearson test. The relationship of SMAC with WLG is confirmed by linear regression analysis (p=0.048), after controlling for other demographic and clinical data; an association of SMAC with female gender (p=0.006) is also observed.

Conclusions: The SMAC questionnaire is a useful tool for understanding the presence of language deficits perceived by patients. Although the questionnaire cannot replace a neuropsychological evaluation, it can assist the clinician in selecting the most appropriate tests to better evaluate language deficits.
Introduction and objectives: The lateral prefrontal cortex is a functionally heterogeneous region that plays a fundamental role in many higher-order cognitive functions including working memory and executive processes, but it is also crucial in planning, organizing, and optimizing behavioral performance based on context (Fuster, 2008; Tanji et al., 2008; Rozzi et al., 2017). In human primates, several motor syndromes occur following lesions of the prefrontal lobe, mainly characterized by the appearance of complex motor behaviors made in an inappropriate context (Lhermitte et al., 1986). In line with this evidence, neuroanatomical studies of the macaque brain, have demonstrated that a specific sector of the ventrolateral prefrontal cortex is interconnected with the parieto-premotor grasping network (Borra et al., 2011, 2017). Accordingly, a recent study of our group revealed that these sectors contain neurons active during planning and execution of grasping actions in different contexts, including a Go/No-Go task instructed by cues (Simone et al., 2015). However, in this latter study have been analyzed the movement-related neurons that represent only a small subset of the task-related neurons (<14%). In the present study, to better assess the role of these prefrontal sectors in linking context and behavior we analyzed the responses of the entire population of recorded neurons during the aforementioned Go/No-Go task.

Results: Our data show that: the instructing cue is encoded in terms of the behavioral outcome it represents, the objects encoding seems to be related more to a pragmatic description of the object rather than a pictorial one, and the activity of neurons active during action/inaction is not strictly related to the general behavioral rule, but to the intrinsic goal of the motor act, namely taking possession of the object.

Conclusions: Our study showed that neurons encoding instructing cue have a crucial role for creating an association between stimuli and responses, hence for association learning.
P47. Characterization of cell assemblies and their organization in the macaque prefrontal cortex
Londei F.1,2, Ceccarelli F.1, Arena G.1, Ferrucci L.1, Di Bello F.1, Genovesio A.1
1 Department of Physiology and Pharmacology, Sapienza University, Rome, Italy; 2 PhD Program in Behavioral Neuroscience, Sapienza University, Rome, Italy

It is well-known that neurons correlate their activity by organizing themselves into larger groups, so-called cell assemblies (Hebb, 1949). These neural superstructures are considered building blocks of higher cognitive functions. Using a recent algorithm for cell assembly detection that allows the automatic selection of the optimal bin and lags associated with each assembly (Russo et al., 2017), we studied the formation of cell assemblies in the macaque prefrontal cortex during the performance of two tasks involving duration and distance discriminations (Genovesio et al., 2012).

For each neuron belonging to an assembly, we extracted from the overall activity (All-Spikes) only those spikes fired during assembly activation (Assembly-Spikes).

We wondered whether being part of an assembly implied a concordance in preferences for a task-relevant variable. Therefore, we investigated the concordance of preferences for the position and the colour of responses between neurons belonging to the same assembly. We found that while the probability that all neurons shared the same preference was close to chance when considering All-Spikes, it increased up to 80% by considering only Assembly-Spikes. Furthermore, focusing on assembly of two cells, we found that the formation of such assemblies occurred more frequently for nearby neurons (neurons recorded by the same electrode) than for farther ones. We also found that their correlation occurred with precise 0-lag synchronization more often for nearby neurons than distant ones. Hence, distant neurons appear to have activities that correlate more sequentially.

Finally, we observed that these assemblies of neighbouring neurons persisted more often between tasks, suggesting that assemblies formed by neighbouring neurons were more robust to context switching.

Our results suggest that this method of analysis has great potential for obtaining information about how neurons interact with each other.
Introduction and objectives: Ultrasonic vocalizations (USV) analysis is a crucial and fundamental point for studying communication between mice. Such analyses can be useful when studying different populations of mice (von Merten et al., 2014), in different social contexts (von Merten et al., 2014; Chabout et al., 2016), and when comparing mice with genetic mutations (Chabout et al., 2016) or under specific treatments (Premoli et al., 2021). Based on previous studies, we examined the USV spectra structure and the correlation between USV and behavior in two groups of wild type mice: vehicle-treated mice (VH) and CBD-oil treated mice (CBD). Previous studies demonstrated that CBD is able to increase social behavior in mice. The aim of the study is to clarify how mice communicate with each other and if their vocalizations have a meaning in the context of social interaction.

Results: After conducting a statistical analysis, a pattern in the number of sounds was revealed: mice of the CBD class were more inclined to produce sounds than those belonging to the VH group. Moreover, a difference was found in the choice of syllables between the two groups. Furthermore, CBD-mice emit sequences with a more complex structure than VH-mice. An analysis of the sounds produced during certain types of behaviors was also made. The study identified three predominant behaviors producing the highest number of sounds for both CBD and VH mice: body sniffing, nose-nose sniffing, and anogenital sniffing. In this experiment, the difference between different classes of mice performing the same behavior and the similarity of sounds for different types of behaviors in mice belonging to different classes were shown.

Conclusions: This work explored the possibility of studying USV and behaviors in mice in order to clarify the role of mice acoustic communication and to understand the effect of a pharmacological drug.
P49. Behavioral correlates of fear memory in an animal model of Post-Traumatic Stress Disorder: Ultrasonic vocalizations and sex differences

Riccardi E., Turchetti L., Morena M., Campolongo P.
Dept. of Physiology and Pharmacology, Sapienza University of Rome, 00185 Rome, Italy; European Center for Brain Research (CERC), Santa Lucia Foundation, 00143 Rome, Italy

Introduction and objectives: Stress exposure may lead to the development of post-traumatic stress disorder (PTSD), a chronic psychiatric disease characterized by over-consolidation, generalization and impaired extinction (Yehuda et al., 2015). The return of fear following the acquisition of fear extinction learning is common (Quirk et al., 2008) and often responsible for the PTSD-treatment failure (Craske et al., 2008; 2014). While only a small proportion of trauma-exposed individuals develop PTSD, women have a twofold greater risk, prevalence and duration of PTSD than men (Breslau, 2009). We have recently demonstrated that there is a link between fear extinction and 22-kHz USV emission in a fear conditioning paradigm, associated to profound sex differences (Riccardi et al., 2021). Thus, in the present study we tested the extent to which 22-kHz USVs would mirror freezing behavior in an animal model of PTSD (Morena et al., 2018), and we examined potential sex differences in fear acquisition and extinction.

Results: Our results indicate that, although during trauma exposure males show higher freezing levels while females exhibit a greater reactivity to trauma, they both emit a similar number of alarm USVs. Moreover, along the extinction sessions, the number of USV emission gradually decreases in both sexes, mirroring the freezing response, although females show a higher extinction rate compared to males, in terms of both freezing and USV. Taking only freezing into account, a single mild FS exposure induces a conditioned fear response in males only and reinstatement in both male and female rats. However, reinstatement of USV emission has been observed only in males.

Conclusions: Taken together, the present findings reveal sex differences in trauma response and extinction process in our model of PTSD and underline the importance of 22-kHz USV analysis, in parallel with freezing, to provide a complete index of fear memory.
P50. Envisioning the future: an ALE meta-analysis of functional neuroimaging studies

Santacesaria P., Scarpazza C., Cona G.

Padova Neuroscience Center (PNC), University of Padua, Via Orus 2/B, 35131 Padua, Italy; Department of General Psychology, University of Padua, Via Venezia 8, 35131 Padua, Italy

Introduction and objectives: Our representations of the future are processed in the service of several different cognitive processes including prospective memory, temporal discounting and episodic future thinking (Schacter et al., 2007; Szpunar et al., 2014). These functions encompass distinct cognitive operations and neural substrates, but they may also share common neural mechanisms since they all operate on the elaboration of time material, particularly future-related. The goal of the present meta-analysis is to understand whether there is a core network underneath future-oriented cognition, using the Activation Likelihood Estimation (ALE) method (Eickhoff et al., 2019). Following the PRISMA guidelines (Moher et al., 2009), a total of 30, 20 and 26 neuroimaging studies were included for future thinking, prospective memory and temporal discounting, respectively.

Results: Results reveal different patterns of brain activation depending on the cognitive function involved. Specifically, two regions of the default mode network were consistently found active across episodic simulation tasks, the posterior cingulate and the medial prefrontal cortices; whereas the encoding of future intentions exhibited a bilateral activation of the insulae. Temporal discounting showed a high convergence activation in the left inferior frontal gyrus.

Conclusions: The conjunction analysis of the three explored domains showed no significant overlap, suggesting that future-related cognitive processes do not share a specific network, but they are function-dependent.

Bibliography:


P51. Exploring the effect on brain health of the dietary glycotoxin Methylglyoxal in aged mice
Department of Molecular and Translational Medicine, University of Brescia, Italy; Center for Neural Science, New York University, New York, United States

Introduction and objectives: Methylglyoxal (MG), a potent glycotoxin that can be found in diet, is one of the main precursors of Advanced glycation end products (AGEs). AGEs mediate their pathological effects by activating signaling cascades mainly via their receptor RAGE. The accumulation of AGEs is implicated in many health disorders including Alzheimer’s disease (AD). It is well known that modifications in lifestyle such as nutritional interventions can be of great value for preventing brain deterioration. Therefore, the aim of this study was to evaluate in vivo how an oral MG treatment, that mimics a high MG dietary intake, could affect brain health.

Results: From Y-maze test in mice, a defect in working memory was observed. MG serum concentration was found significantly higher in MG-mice. The gene and protein expressions of RAGE were found remarkably increased in the hippocampus of MG mice, an area where the activity of glyoxalase 1 was instead found reduced. Furthermore, at hippocampal level, MG mice showed increased expression of proinflammatory cytokines (IL-1β, IL-6) and increased activities of NADPH oxidase and CAT. MG administration also increased the gene and protein expressions of Presenilin-1, a subunit of the γ-secretase protein complex linked to AD.

Conclusions: These findings suggest that high MG oral intake induced alteration directly in the brain and might established an environment predisposing to AD-like pathological conditions. Encouraged by these results, we would like to investigate if MG could exacerbate the effects induced by Scopolamine, a drug widely used to resemble AD-like neuropathological features in mice in order to understand if dietary MG intake could contribute as an additional modifiable risk factor to cognitive decline.
POSTER SESSION
Development
P52. Early life stress exposure: a pattern of adaptation or vulnerability to the development of stress-related psychopathologies?

Di Cesare B., Milione A., Morena M., Campolongo P.

Dept. of Physiology and Pharmacology, Sapienza University of Rome, 00185 Rome, Italy; European Center for Brain Research (CERC), Santa Lucia Foundation, 00143 Rome, Italy

Introduction and objectives: Stressful experiences in childhood are among the most important risk factors for the development of a whole spectrum of mental and physical illnesses in adulthood (Heim et al. 2012). The plasticity of the brain is particularly pronounced in the early stages of life; therefore, aversive conditions can have conspicuous and long-lasting effects during early development. Here we assessed whether prenatal stress (PNS) exposure could confer susceptibility or resilience to the development of psychopathological conditions in adolescence following a second stressful experience in adolescence (single prolonged stress, SPS) and whether these alterations were sex-specific in rats. Behavioral alterations in the emotional and cognitive domains were examined in adolescent rats of both sexes exposed to PNS (at gestational day 14) or SPS (at postnatal day 23) or both (Marchisella et al. 2021; Mancini et al. 2021) in a battery of tests.

Results: Our results in males showed behavioral alterations in the Open field following exposure to PNS and alterations of social play behavior in the PNS or SPS alone groups, as compared to controls. Furthermore, exposure to both stressors reduced auditory conditioned fear acquisition and recall in males only. Exposure to PNS or SPS increased pre-pulse inhibition, while it did not alter the total amplitude of a startle response, in males. Overall females remained largely unaffected by the adverse exposures only showing increased grooming behavior in the Open field task following exposure to the PNS+SPS relative to controls, possibly indicating increased anxiety-like behavior.

Conclusions: Taken together, these results suggest that early life stress alters emotional and cognitive behavior in adolescence and lay the foundations for more mechanistic investigations to allow the identification of new prophylactic and therapeutic targets for the treatment of psychopathologies at a young age.
POSTER SESSION
Excitability, synaptic transmission, network functions
P53. Sub-toxic doses of glyphosate cause synaptic alterations in inhibitory transmission
Chiantia G.1, Gurgone A.1, Franchino C.2, Marcantonii A.2, Giustetto M.1
1Department of Neuroscience, University of Turin, Turin (Italy); 2Department of Drug Science and "NIS" Inter-departmental Centre, University of Turin, Turin (Italy)

Introduction and objectives: Glyphosate (Gly) is a broad-spectrum herbicide known to inhibit the enzyme 5-enolpyruvylshikimate-3-phosphate synthase. This latter is expressed in plants but not in mammals and other vertebrates, making its use in humans plausible (Dechartres et al. 2019). In 2017, EC deliberated for an extension of the current maximum limits of exposure to Gly for another 5 years setting as acceptable daily intake (ADI) the concentration 0.5 mg/kg body weight per day (Santovito et al. 2018). However, little is known about several aspects of the neurotoxic outcome of Gly. Chronic in-vivo treatment with toxic doses of Gly-based herbicides, produced a reduction of serotonin, dopamine, norepinephrine, and acetylcholinesterase contents and increased oxidative stress in several parts of the CNS, associated with increased anxiety and depression-like behaviors as well as learning and memory impairments (Ait Bali et al. 2019). Despite the compelling evidence pointing to a neurotoxic effect of Gly, a detailed mechanistic description of how Gly could affect synaptic transmission is lacking. To fill this gap, in this study we performed MEA and whole-cell patch-clamp experiments in the voltage-clamp configuration in cultured mouse hippocampal neurons.

Results: We found that 30 min treatment with Gly at the ADI concentration did not produce any alteration either in spontaneous network activity or in the evoked excitatory postsynaptic currents. Instead, Gly reduced the amplitude of evoked inhibitory postsynaptic currents, as a consequence of a decreased number of release sites and quantal size. Moreover, multiple probability fluctuation analysis and cumulative amplitude analysis of eIPSCs showed a reduced size of the synchronous readily releasable pool. Finally, the peak-scaled variance analysis of miniature IPSCs revealed fewer postsynaptic GABAA receptors in Gly treated neurons.

Conclusions: These data unveil that subtoxic doses of Gly produce impairment of inhibitory transmission by affecting both pre- and postsynaptic mechanisms.

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Introduction and Objectives: Epidemiological studies show that the risk of epilepsy is increased in Alzheimer’s Disease (AD) patients. Concurrently epileptic patients have an elevated risk to develop dementia, suggesting that hippocampal epileptiform activity might be involved in AD onset and progression (Pandis et al., 2012; Vossel et al., 2013). Epileptic-like seizures are related to network alterations and aberrant γ-oscillations - due to parvalbumin interneuron dysfunctions - resulting in brain hyperexcitability (Palop et al., 2007; Cardin et al., 2009; Verret et al., 2012). Although the causes of epileptiform activity are still unclear, many studies reported the crucial role of Dopamine (DA) and its receptors in the modulation of hippocampal network activity (Costa et al., 2016). Interestingly, since clinical and experimental studies show that dopaminergic neurodegeneration in the Ventral Tegmental Area (VTA) is one of the precocious events in AD (Nobili et al., 2017; De Marco & Venneri, 2018; Sala et al., 2021), we hypothesized that a reduction of dopaminergic tone in the projection areas could trigger brain hyperexcitability and related dysfunctions. To investigate whether DA loss might affect network integrity, we characterized the mechanisms underlying hippocampal hyperexcitability in the Tg2576 model of AD at different ages – before, at onset and at advanced DA neuron degeneration.

Results: Our data reveal that, progressively with VTA degeneration, CA1 pyramidal neurons exhibit increased epileptiform activity and reduced GABAergic inputs, due to decreased parvalbumin interneuron excitability. We also show a lower number of parvalbumin interneurons, associated with an impaired p-CREB expression, a pro-survival transcriptional factor. Importantly, boosting dopaminergic transmission with L-DOPA in Tg2576 mice can rescue the GABAergic tone onto CA1 neurons, ameliorating hippocampal hyperexcitability.

Conclusions: These results suggest that precocious VTA neurodegeneration and DA depletion may lead to hippocampal hyperexcitability, revealing a possible mechanism at the basis of epileptiform activity in early AD.
The biochemical and mechanical properties of the actin cytoskeleton drive a large range of cellular processes in animal cells including polarity, morphogenesis, and motility. In neurons, the ability of spines to undergo rapid morpho-functional changes following activity is fundamental for plasticity and memory formation. The WAVE regulatory complex (WRC) is one of the most important component that regulates actin polymerization. One member of this complex is Cytoplasmic FMRP Interacting Protein 2 (CYFIP2), originally identified as a FMRP interactor. Interestingly, CYFIP2 transcript undergoes RNA editing, a post-transcriptional mechanism catalyzed by ADAR enzymes, that leads adenosine (A) to inosine (I) deamination. When this reaction occurs in the coding sequence it causes an aminoacid substitution that contributes to increase the proteomic complexity of the cells. CYFIP2 editing results in a K/E substitution at amino acid 320 in both human and mouse proteins but its functional meaning is still unknown. In this study, we aim at investigating the potential implication of this process related to actin dynamics during neuronal development and synaptogenesis. Our results show that CYFIP2 RNA editing reaction is active only in the central nervous system and specifically regulated in different brain areas. CYFIP2 RNA editing level increases during neural development and may be regulated by neuron activity. Further, preliminary results show functional differences of the edited and unedited CYFIP2 proteins in regulating cellular migration. Our data, although preliminary, may contribute to unveil the functional role of RNA editing on the CYFIP2 protein and in turn on neuronal function.
**P56. Regionally specific extracellular matrix in the central nervous system of the mouse**

Moretti M.¹, Baldassarro V. A.¹,², Lorenzini L.¹,²  
¹Department of Veterinary Medical Sciences, University of Bologna, Italy; ²Health Science and Technologies Interdepartmental Center for Industrial Research (HST-ICIR), University of Bologna, Italy

**Introduction and objectives:** The extracellular matrix (ECM) is produced by resident cells of central nervous system (CNS) and plays a fundamental role in the structure and functions of the different areas, as well as in physiological and pathological processes. In this study we investigated the loco-regional differences of the ECM in adult mouse CNS by analyzing mRNA expression level of about 100 ECM-related genes in five different areas: cerebral cortex (CTX), cerebellum (CB), hippocampus (HIP), basal ganglia (BG) and spinal cord (SC). A bioinformatic approach was used for comparison between areas, and resulting data were correlated to the cell composition.

**Results:** We used a combination of gene array profiling of the ECM (RT² qPCR Arrays) and single gene RT-PCR for CNS-ECM coding genes. By a bioinformatic analysis, the five areas were clustered into two families, one including BG, CTX, and HIP, the other including CB and SC. These differ for the white/grey matter (WM/GM) ratio, being the first mainly composed by GM, and the second by WM. This was also confirmed at molecular level by the different expression level of mRNA encoding for markers of the four principal cellular types in CNS (Eno2 for neurons, Gfap for astrocyte, Pdgfra for oligodendrocyte precursor cells and Mbp for oligodendrocytes) which indicates Eno2 expression in CTX more than 100-times compared to SC where is hardly detectable, while Gfap and Pdgfra are comparable in all areas. Mbp is highly expressed in SC and BG. Finally, the STRING software analysis, allowing the analysis of the interaction of the proteins encoded by the analyzed genes and their functional clustering, indicate that osteopontin and trombospondin1 are the core in the WM-enriched area, while the perineuronal net protein netrin4 in the GM-enriched areas.

**Conclusions:** The molecular analysis offers a useful tool for the study of regionally specific composition of the ECM.
POSTER SESSION
Novel Methods and Technology Development
P57. Gamma transcranial alternating stimulation in Alzheimer’s disease: a randomized, double-blind, sham-controlled, crossover study

Cantoni V.1, Benussi A.1,2, Grassi M.3, Brechet L.4, Michel C. M.4,5, Datta A.6, Thomas C.6, Gazzina S.7, Cotelli M. S.8, Bianchi M.8, Premi E.9, Gadola Y.1, Cotelli M.10, Pengo M.11, Perrone F.1, Scolaro M.7, Archetti S.12, Solje E.13,14, Padovani A.1,2, Pascual-Leone A.15,16,17, Borroni B.1,2

1Neurology Unit, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy; 2Neurology Unit, Department of Neurological and Vision Sciences, ASST Spedali Civili, Brescia, Italy; 3Department of Brain and Behavioural Sciences, Medical and Genomic Statistics Unit, University of Pavia, Pavia, Italy; 4Functional Brain Mapping Laboratory, Department of Fundamental Neuroscience, University of Geneva, Geneva, Switzerland; 5Center for Biomedical Imaging (CIBM), Lausanne, Switzerland; 6Research & Development, Soterix Medical, Inc., New York, USA; 7Neurophysiology Unit, Department of Neurological and Vision Sciences, ASST Spedali Civili, Brescia, Italy; 8Neurology Unit, Valle Camonica Hospital, Esine, Brescia, Italy; 9Stroke Unit, Department of Neurological and Vision Sciences, ASST Spedali Civili, Brescia, Italy; 10Neuropsychology Unit, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia; 11Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy; 12Clinical Chemistry Laboratory, Diagnostic Department, ASST Spedali Civili Brescia, Brescia, Italy; 13Institute of Clinical Medicine, Neurology, University of Eastern Finland, Kuopio, Finland; 14Neuro center, Neurology, Kuopio University Hospital, Kuopio, Finland; 15Department of Neurolgy, Harvard Medical School, Boston, MA, USA; 16Hinda and Arthur Marcus Institute for Aging Research and Deanna and Sidney Walk Center for Memory Health, Hebrew SeniorLife, Boston, MA, USA; 17Guttmann Brain Health Institut, Barcelona, Spain

Introduction and objectives: Alzheimer’s disease (AD) is characterized by progressive memory impairment which seems to be preceded by a dysfunction of cerebral high frequency oscillations, particularly in the gamma frequency band. (Babiloni et al., 2016).

The clinical potential of restoring gamma oscillations with non-invasive brain stimulation has recently gained attention. In this context, tACS is a unique, noninvasive, easy to apply, safe, painless, inexpensive tool which allows the modulation of brain rhythms (Vosskuhl et al., 2018). We carried out a randomized, double-blind, sham controlled, crossover study to assessed whether non-invasive brain stimulation with transcranial alternating current stimulation at gamma-frequency (γ-tACS) applied over the precuneus can improve episodic memory and modulate cholinergic transmission by modulating cerebral rhythms in early AD.

Results: We observed a significant improvement in the Rey auditory verbal learning (RAVL) test immediate recall (p<0.001) and delayed recall scores (p<0.001) after γ-tACS but not after sham tACS. Face-name associations scores improved with γ-tACS (p<0.001) but not after sham tACS. Short latency afferent inhibition, increased only after γ-tACS (p<0.001). ApoE genotype and baseline cognitive impairment were the best predictors of response to γ-tACS. Clinical improvement correlated with the increase in gamma frequencies in posterior regions and with the amount of predicted electric field distribution in the precuneus.

Conclusions: The restoration of gamma oscillations in AD can safely and efficiently induce entrainment of neural oscillations, improving episodic memory functions and ameliorating cholinergic deficits. The refinement of predictors of outcome may best identify patients who may benefit most from γ-tACS stimulation. These findings suggest that γ-tACS stimulation over the precuneus may represent a novel therapeutic approach in AD (Kim at al., 2021). Future studies with multisession γ-tACS and with at-home setting design are warranted (Bréchet et al., 2021).
P58. Study of the brain extracellular spaces using expansion microscopy.
Joshi R.1, Viggiano D.2,3
1Department of Clinical and Experimental Medical Sciences, University of Campania “Luigi Vanvitelli”, Naples;
2Department of Translational Medicine, University of Campania “Luigi Vanvitelli”, Naples;3BIOGEM, Ariano Irpino.

Introduction and objectives: Brain extracellular spaces surround the cells of central nervous system. They are essential for providing ions necessary for neuronal activities and intercellular communication. They are studied by Electron or Super-resolution microscopy, MRI-DTI etc. In this communication we have tested the use of Expansion Microscopy to characterize brain extracellular spaces. Expansion microscopy (Viggiano et al. 2018) is a recently developed technique that deals with sample expansion using a polymer system. This technique is useful in visualizing the samples at high magnification with intricate details by just observing expanded samples under a simple light microscope. The details obtained using this technique are not seen by conventional light microscope. It gives better magnification and hence eliminates the usage of expensive and time & space-consuming microscopes. Expansion microscopy is currently limited to the expansion of only fluorescent labeled samples. But, doctors who are handling numerous patient samples cannot use complex systems like fluorescent labeling and expensive microscopes daily. Therefore, we aim to develop a technique, where common histological staining methods can be used with expansion microscopy. So, we have developed a polymer system that can be used for the expansion of brain tissues.

Results: The technique of expansion microscopy allows for an optimal visualization of brain extracellular perineuronal spaces. After the expansion, these spaces are up to 2 micron thick and can be easily quantified. The size of the nuclei was enlarged by 5-6 times the original size.

Conclusions: Our implementation of the Expansion microscopy to study the brain extracellular spaces allows for a simple quantification of these brain structures. This technique can be a boon to doctors in analyzing the brain tissues in more details.

Bibliography:

POSTER SESSION
Sensory and motor systems
P59. Cerebellar climbing fiber activity can reshape the structure of the olivocerebellar circuit

Bergamini M.\textsuperscript{1,3*}, Musto M.\textsuperscript{1,2*}, La Terra A.\textsuperscript{3}, Marte A.\textsuperscript{3}, Benfenati F.\textsuperscript{1,2,3}, Grasselli G.\textsuperscript{1,2,4}

\textsuperscript{1} Center for Synaptic Neuroscience, Istituto Italiano di Tecnologia, Genoa, Italy; \textsuperscript{2} IRCCS Ospedale Policlinico San Martino, Genoa, Italy; \textsuperscript{3} Department of Experimental Medicine, University of Genoa, Genoa, Italy; \textsuperscript{4} Department of Pharmacy, University of Genoa, Genoa, Italy. * equal contribution.

Introduction and objectives: Cerebellar climbing fibers (CFs) convey a teaching signal to Purkinje cells (PCs) that is crucial for learning. It is known that CFs can undergo activity-dependent synaptic plasticity (Hansel & Linden, 2000). However, although they are known to be able of major lesion-induced structural plasticity, it is still not clear whether they are able of structural modifications dependent on their activity during adulthood and how this affects the architecture and function of the olivocerebellar circuit (Nishiyama 2014). Here we investigate whether CF activity controls the morphology of the fiber itself and of its target PC, as well as the underlying molecular mechanism.

We chronically reduced CF intrinsic excitability in mouse in vivo by knocking-down voltage-gated sodium channels (NaV1.1 and NaV1.2) or the growth-associated protein GAP-43 with lentiviral vectors and used immunofluorescent staining, confocal microscopy and 3D reconstructions to assess morphological effects on CF and PC morphology.

Results: We observed that knocking-down NaV1.1/2 causes a CF atrophy (affecting its length and branching), inducing a compensatory increase of density of synaptic terminals as well as of PC dendritic spines. Knocking-down GAP-43 had a similar effect on CF branches but not on synaptic terminals or PC spines suggesting that it may mediate activity-dependent CF structural plasticity and its associated compensatory increase in synaptic terminals through different mechanisms.

Conclusions: Here we showed that CFs can undergo activity-dependent structural plasticity affecting PC morphology, potentially affecting the circuit function, and suggests that this may be mediated by GAP-43.

Bibliography:


P60. Inferring brain-wide circuit modules linking structural and functional connectivity in zebrafish larvae

**Bruzzone M.**, Manjunatha K. K. H., Nicoletti G., Suweis S., Dal Maschio M.

1Padua Neuroscience Center - PNC, University of Padova, Padova, Italy; 2Laboratory of Interdisciplinary Physics, Department of Physics and Astronomy, University of Padova, Padova, Italy; 3Department of Biomedical Sciences, University of Padova, Padova, Italy

**Introduction and objectives:** How neuronal circuits support the processing of the sensory information and the coordination of an appropriate motor outcome is still an open challenge. In zebrafish larvae, optical methods allow for the reconstruction of the activity from several thousand neurons across the entire brain in parallel in response to a sensory stimulation (Bruzzone et al., 2021). This kind of datasets provides an ideal workbench for the application of theoretical approaches aiming at generating plausible circuit models where to test hypotheses on the circuit mechanisms, to assess the network properties and its functional organization (Triplett et al., 2020). This approach is also taking advantage from everyday-more-detailed reconstructions of the neuronal wiring diagrams and connectomes whose layouts offer a sort of structural blueprint underlying the circuit activity (van der Plas et al., 2021). However, it’s not yet clear if and to what extent the patterns extracted from the connectome reconstruction can explain the observed functional information, and viceversa. Here, we address these aspects.

**Results:** We started with the generation of a structural connectome from the publicly available dataset of single cell reconstructions (Kunst et al., 2019) to characterize the network properties of the fish brain. An algorithm for graph analysis was implemented to reveal distinct circuit modules based on the relative connection strength between cells. We are currently comparing the structure of the identified modules and the activity recorded from neurons, both registered into the same anatomical space. This method could represent a valid approach for the analysis of the brain functioning and its underlying organization.

**Bibliography:**


P61. Elusive effects of Hydrogen Sulphide in mouse spinal cord cultures

De Stefano S.1,4, Ciaiola F.1,4, Salvatori I.2,4, Ferri A.3,4, Valle C.3,4, Mercuri N. B.1,4, Chiurchiù V.3,4, Spalloni A.3, Longone P.4

1Department of System Medicine, University of Rome “Tor Vergata”; 2Department of Experimental Medicine, Faculty of Medicine, University of Roma “La Sapienza”, Rome, Italy; 3Institute of Translational Pharmacology (IFT), National Research Council (CNR), Rome, Italy; 4Fondazione Santa Lucia IRCCS, Rome, Italy.

Hydrogen sulphide (H2S) is a colourless gas with an unpleasant odor considered a toxic environmental pollutant. It is now recognized as an important endogenous neuromodulator (Peers et al., 2012) owning protective effects, as established in “in vivo” models of Parkinson’s and Alzheimer’s diseases (Giuliani et al., 2013). In Amyotrophic Lateral Sclerosis (ALS), a lethal disease characterized by the progressive degeneration of the upper and lower motor neurons (MNs), we have reported poisonous levels of H2S in the liquor of patients (Davoli et al., 2015). Furthermore, we and others have described increased neuronal death and the recruitment of death-inducing pathways (apoptosis and necrosis) (Greco et al., 2018) in the presence of H2S, even at physiological concentrations (Cheung et al., 2007). Finally, we have demonstrated that the pharmacological inhibition of H2S production is able to increase lifespan in SOD1G93A female mice (Spalloni et al., 2019).

The aim of the present study was to further evaluate the effects of NaHS (200 micromolar), an H2S donor, in mouse mixed spinal cord culture at different times of incubation (3, 6, and 18 hours). We show that H2S induces (i) the release of cytochrome C; (ii) the increase of the levels of caspase 3; (iii) decreases ATP production and mitochondrial respiration rate; (iv) causes mitochondrial depolarization. Furthermore, on one hand H2S is able to increase the expression of connexin 43 protein, a strong indicator of astrocytic gap-junction expansion, a phenomenon that has been linked to the ALS-related motor neuron loss (Almad et al., 2016). On the other hand, it shows also the ability to revert microglia polarization from M1 to M2, which is observed through an increase in CD206 (anti-inflammatory) and a decrease in CD86 and CD68 (pro-inflammatory). The ambiguous effects of H2S on the cellular and molecular homeostasis in mixed spinal cord culture and its potential role in ALS will be discussed.
P62. When right goes left: phantom touch induced by mirror box procedure in healthy individuals
Cirillo E.¹, Gammeri R.¹, Caldano M.¹, Sabatelli I.¹, Cesim E.¹,², Salatino A.¹,³, Berti A.¹, Ricci R.¹
¹Department of Psychology, University of Turin, Turin, Italy; ²Dokuz Eylul University, Izmir, Turkey; ³Institute of Neuroscience (IoN), Université Catholique de Louvain, Brussels, Belgium

Introduction and objectives: We recently reported a new phenomenon in which post-stroke patients erroneously responded to bilateral asymmetrical stimulations on the dorsum of the hands as if they were touched on symmetrical position (Ricci et al., 2019). That is the phenomenal experience of touch can be non-veridical in brain-damaged patients. We called this phenomenon ‘synchiric extinction’, since it resembled synchiria, but exclusively arose during simultaneous touch. Here, we investigated the possibility of inducing phantom tactile sensations in healthy individuals. To this aim, we used the Tactile Quadrant Stimulation (TQS) test in which subjects must indicate where they perceived a double tactile stimulation applied simultaneously in different quadrants on the dorsum of the two hands. The task was performed with the left-hand out of sight and the right-hand reflected in a mirror, so that the right hand looks like the own left hand.

Results: Non-parametric analyses of tactile errors (i.e., synchiric extinction, mislocalization and extinction) showed that participants made significantly more errors (p<.0001) in the Mirror Conditions compared to the Baseline Condition. In particular, in the crucial condition, synchiric extinction was significantly greater (p < .0001) than other types of possible errors (i.e., mislocalization and extinction).

Conclusion: In the crucial condition, the vision of the stimulated right hand reflected in the mirror, elicited on the left hand, that was touched in a different quadrant, the sensation of having been touched in the same quadrant as the right hand. In other words, we found in healthy subjects the same phantom touch phenomenon that we found in patients. We interpreted these results as a modulation of tactile representation by bottom-up (multisensory integration of stimuli coming from the right real and the right reflected hand) and possibly top-down processing (body ownership distortion) triggered by our experimental setup.

Bibliography:

Introduction and objectives: The olfactory bulb (OB) is the first center of odor processing. In the forebrain, it hosts the most numerous dopaminergic (DA) population, which can be identified by the expression of tyrosine hydroxylase (TH). TH+ cells are inhibitory interneurons classified into two major categories based on their morphology and generation time, but their function is still unclear. We combined mouse genetics, adeno-associated viral vectors (AAVs) delivery and advanced imaging techniques, we aim at investigating how each dopaminergic subpopulation processes ethologically-relevant odors.

Results: We took advantage of two different approaches to label the OB DA population: a transgenic mouse line driving GFP expression under TH promoter, and direct OB delivery of AAVs expressing TH-Cre together with a Cre-dependent fluorescent reporter (tdTomato). We first validated the specificity of TH-cre labeling in TH-GFP mice. Our quantitative analysis shows that the proportion of tdTomato and GFP-expressing cells is ~90%. Moreover, in accordance to literature, we found that the percentage of tdTomato+ cells immunolabeled for TH is ~60%, while no co-expression has been detected with calretinin or calbindin. To specifically label early-born DA cells, we injected TH-Cre AAVs in P0 mice and a Cre-dependent GCaMP reporter, which can be used as a morphological marker and functional indicator of cell activity, in adult age. Our data confirm the selectivity of this paradigm to label the early-born DA cells. Currently, we are setting up two-photon imaging experiments to characterize the responses of TH+ cells to sexually-relevant odors.

Conclusions: Our results show that we can specifically label OB dopaminergic cells according to their birth date and morphological features. By using this approach, we plan to perform functional imaging on different DA populations to identify their contribution in processing ethologically-relevant olfactory cues.