epilepinet

Epileptogenesis and Epilepsy Network

2nd Annual Meeting

- Book of abstracts -

Lisboa, Portugal - moved to zoom

10th - 11th Jan, 2022

\ FUNDING:

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 952455

\ PARTNER INSTITUTIONS:

Instituto de Medicina Molecular João Lobo Antunes

LUNDS UNIVERSITET

SAPIENZA UNIVERSITÀ DI ROMA

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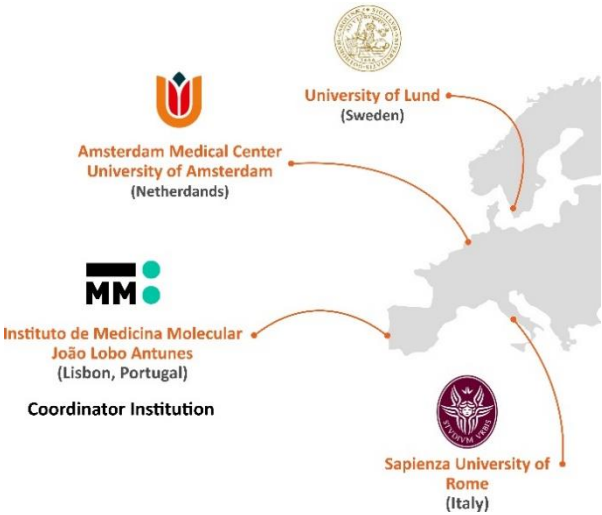
EPILEPTOGENESIS AND EPILEPSY NETWORK:

from genes, synapses and circuitries to pave the way for novel drugs and strategies

EPIEPINET is an European Consortium funded by the European Union that includes four leading institutions in the field of epilepsy research:



OUR MISSION:

"Promote collaborative multidisciplinary & translational research in epilepsy"

"Promote public awareness on epilepsy caregiving and research to patient communication"

OUR AIMS:

- Increase scientific and technological innovation in epilepsy research by interchange of ideas and researchers among partners
- Promote joint grant application and joint training of PhD students
- Train young researchers and promote their international career
- Increase the awareness on epilepsy among caregivers, patients & society

OUR STRATEGIES:

- Organise scientific meetings
- Organise thematic hands-on workshops and summer schools
- Promote short-term & on-site training visits for scientific and technology transfer between partners
- Promote community-oriented debates & dissemination material

OPPORTUNITIES FOR EARLY-STAGE RESEARCHERS:

Either by (1) attending **Summer Schools**, **Workshops**, **Annual Meetings** and **Conferences** or by (2) **performing a scientific / technical short-term mission in another partner institution**, EPIEPINET can help you to:

- establish novel collaborations that boost your research projects
- learn new techniques or perform part of your research in another partner institution within a **fully funded short-term mission**
- find new opportunities for the next steps in your career
- improve your CV by promoting internationalization of your career

This Summer School is a great opportunity to network and find out all the oportunities that Epiepinet has to give you.

FOR MORE INFORMATION:

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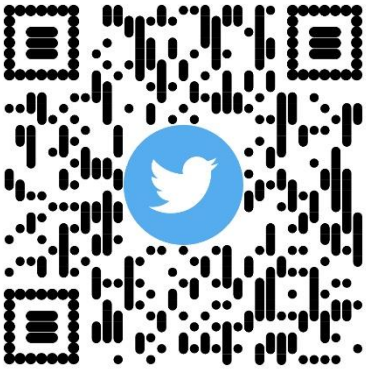

#EpiEpiNet

(Scan the QR code to access the links)

\ WELCOME BY THE ORGANISING TEAM

The organising team would like to welcome you to Lisbon and to the 2nd EpiEpiNet Annual Meeting (the first one in-person).

The aim of this meeting is to discuss research projects and boost scientific interaction among EPIEPINET partner thus paving the way towards upcoming joint projects and short-term missions.

We wish you a fruitful Meeting, both personally and scientifically.

For any questions during the event just get in contact with us:
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\ PRACTICAL INFORMATION

ABOUT LISBOA ORIENTE & PARQUE DAS NAÇÕES

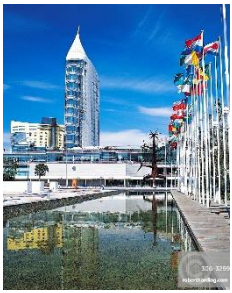
Located in East border of Lisbon, Parque das Nações is one of the most modern neighbourhoods in the city.

Most of its area used to be an abandoned hydroport and industrial plants until the end of the XX century. In the late 1990s, this area suffered a dramatic intervention and reconstruction to hold the Expo '98 (1998 Lisbon World Exposition).

The Expo'98 hosted exhibitions from 143 countries participated under the umbrella subject of "The Oceans: A Heritage for the Future". The Expo'98 also served as commemoration for the 500th anniversary of the arrival of Vasco da Gama navigator to India 1498, which meant a revolution in World Trading by sea.

After the Expo'98 most of the buildings were repurposed and now Parque das Nações holds many different services, outstanding architecture and gardens which stand along 5 Km of the margin of the Tagus River.

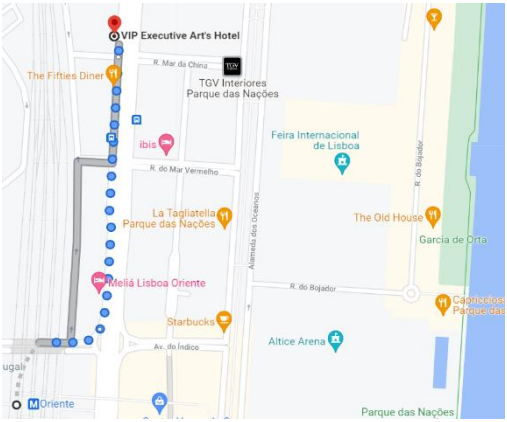
We hope that, apart from sharing great science, you enjoy your time in Lisbon.



VIP Executive Art' Hotel is located at Parque das Nações and can be easily reachable from Lisbon outsides, Lisbon City Centre and Lisbon International Airport.

To reach the Venue by public transportation you can take the train to "Lisboa – Oriente" or the metro to redline station "Oriente". VIP Executive Art' Hotel is only about 5 minutes walking from both train and metro stations.

From the airport, you can easily reach VIP Executive Art' Hotel by car (~5 minutes driving) or by metro (~15 minutes to metro station "Oriente").



WHAT? WHERE?

- **Talks:** Art's Conference Room – 1st floor
- **Group Discussion:** **Group I –** Art's Room – 1st floor
  Group II – Diamante Room – Ground floor
  Group III – Diamante Room – Ground floor
- **"In person" poster hanging:** Art's Room – 1st floor
- **Lunch (10/01): 1st floor Restaurant (next to Art's Room)**
- **Coffee Breaks: 1st floor hall (next to Art's room)**

COVID-19 CONTROL MEASURES

- **Use of face mask is mandatory in closed spaces during the entire duration of the event, except during meals**
- During meals, please respect the maximum number of people per table mentioned by the Hotel staff.
- **Please inform the organization on the results of your COVID-19 tests before the event.**
- If you have not done a COVID-19 test in the 72h (for RT-PCR testes) or 48h (for antigen tests) before your arrival to the event, please inform the organisers. We will provide you with a self-test.
- **If you have any symptoms compatible with COVID-19, please inform the organisers. We have a contingency plan.**

At Epiepinet, we are committed to ensure that the event is a great opportunity to network in a safe and friendly environment

\ PROGRAMME SUMMARY – UPDATED FOR ONLINE

Programme in Western Europe Time zone (Lisbon Time)

For Amsterdam, Rome or Lund time add 1 hour more to the schedule

DAY 1 – MONDAY, 10TH OF JANUARY 2022 (MORNING)

9:00 – 9:15	Welcome Session
9:15 – 10:00	**Short Scientific Communications |** part 1
	Catarina Lourenço | Exploring adenosine augmentation therapies: Adenosine Kinase as a new target in Rett Syndrome
	Diogo Lourenço | Uncovering Cannabidivarin and its neurogenic potential as a novel target for Rett Syndrome
	Hester Meeusen | A novel ketogenic diet slows down seizure development in the rapid kindling rat model of epileptogenesis
10:00 – 10:15	**Short Break**
10:15 – 11:00	**Short Scientific Communications |** part 2
	Vera Neves | Peptide shuttles for receptor independent transport across the BBB
	Gabrielle Ruffolo | Cytokines as potential modulators of neurotransmission in drug-resistant epilepsy: a potential beneficial effect of IL-10
	Anwesha Gosh | Selective inhibition of synaptic transmission by MRS5474 in epileptic rats but not in control ones
11:00 – 11:15	**Short Break**
11:15 – 12:00	**Short Scientific Communications |** part 3
	Katiuscia Martinello | The Neuron Hunters: a 18 years long story spent on human brain tissue
	Mariana Sottomayor | The puzzle: absence seizures, astrocyte complexity and memory impairments
	Rita Soares | The relevance of Mitochondrial Dynamics in Neural Stem Cell Fate
12:00 – 13:00	**Lunch Break**

Programme in Western Europe Time zone (Lisbon Time)

For Amsterdam, Rome or Lund time add 1 hour more to the schedule

DAY 1 – MONDAY, 10TH OF JANUARY 2022 (AFTERNOON)

13:00 – 13:30	**Presentations on planned / potential short-term missions**
13:30 – 14:45	**Group Discussion** | **part 1** | *Breakout rooms* **Group I** – Synaptic Dysfunction & Excitability in Epilepsy **Group II** – Neuroinflammation & Glial Cells in Epilepsy **Group III** – Novel therapies & Developmental epilepsies
13:30 – 13:45	**Breakout room introduction**
13:45 – 14:45	**Online E-poster session**
14:45 – 15:15	**Coffee Break**
15:15 – 17:30	**Group Discussion** | **part 2** | *Breakout rooms*
15:15 – 16:00	**Share of technical skills and resources** *"What can I give? What can I get?"*
16:00 – 16:45	**Common Scientific Interests and future joint projects** *"How can we boost each other's research and careers?"*
16:45 – 17:30	**Time for informal side meetings** (online)

Programme in Western Europe Time zone (Lisbon Time)

For Amsterdam, Rome or Lund time add 1 hour more to the schedule

DAY 2 – TUESDAY, 11TH OF JANUARY 2022

9:30 – 10:30	**Group Discussion** | **part 3** | *Breakout rooms* **Common Scientific Interests and future joint projects** *"How can we boost each other's research and careers?"*
10:30 – 11:00	**Coffee break**
11:00 – 11:45	Presentation of Conclusions from Working Group Discussions (Plenary Session – VIP Art's Room)
11:45 – 12:15	Final Discussion Meeting (Plenary Session – VIP Art's Room)
12:15 – 14:00	**Lunch Break**
14:00 – 14:45	**Executive Council, Governing Council and External Advisory Board Meeting**

\ SHORT SCIENTIFIC TALKS

\ Exploring adenosine augmentation therapies: Adenosine Kinase as a new target in Rett Syndrome

Catarina Miranda-Lourenço[1]

Ana M. Sebastião[1], Maria José Diógenes[1]

[1] Instituto de Farmacologia e Neurociências, Faculdade de Medicina e Instituto de Medicina Molecular João Lobo Antunes, Universidade de Lisboa, Portugal;

Abstract:

Rett Syndrome (RTT) is a rare neurodevelopmental disorder primarily caused by mutations in the methyl-CpG binding protein 2 (MECP2) gene. MeCP2 is known to modulate the expression of brain-derived neurotrophic factor (BDNF), a neurotrophin with essential functions in cell differentiation, synaptic plasticity and survival. BDNF signalling is impaired in RTT. Thus, therapeutic strategies designed at delivering BDNF to the brain could be a breakthrough in RTT treatment. However, this strategy is challenged by the inability of BDNF to cross the blood-brain barrier. Adenosine (ADO) is a neuromodulator that acts mainly through A_1 and A_{2A} receptors (A_1R, $A_{2A}R$). The activation of A2AR potentiates BDNF synaptic actions, important to overcome cognitive deficits presented by RTT patients. On the other hand, A_1R activation provides potent seizure control important to ameliorate epilepsy in RTT patients. Thus, activation of both ADO receptors could be a potential therapeutic strategy.

Previous data obtained by our group showed that targeting the adenosinergic system could be beneficial. Thus, 5-6 weeks old $Mecp2^{-/y}$ animals were administered intraperitoneally with an adenosine kinase (ADK) inhibitor drug, 5-iodotubercidin (ITU). This drug allows an increase in adenosine levels by inhibiting its metabolization.

Through the study of ITU administration, carried out *in vivo*, it was possible to observe a recovery of the effect of BDNF upon LTP potentiation, in electrophysiological recordings performed in hippocampal slices, as well as a recovery of protein levels of TrkB-FL receptors in hippocampal homogenates from ITU-treated $Mecp2^{-/y}$ animals.

Overall, the positive data obtained regarding the reversal of some deficits present in the animal model studied, through the pharmacological inhibition of ADK, reinforces the

importance of adenosine system involvement while suggesting the increase of adenosine levels as a strategy to be explored in RTT.

\ Uncovering Cannabidivarin and its neurogenic potential as a novel target for Rett Syndrome

Diogo M. Lourenço[1,2],
Svitlana Zavalko[1,2], Rita Soares[1,2], Joana M. Mateus[1,2], Rui S. Rodrigues[1,2], Ana M. Sebastião[1,2], Francisco M. Mouro[1,2], Maria José Diógenes[1,2], Sara Xapelli[1,2]

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[2]Instituto de Medicina Molecular | João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal;

Abstract:

Rett Syndrome (RTT) is a rare progressive neurodevelopmental X-linked disorder, caused mostly by mutations in MECP2. This transcriptional and epigenetic regulator has been proposed to modulate neurogenesis and neuronal development, processes known to be affected in both RTT patients and mouse models. Cannabidivarin (CBDV) a non-psychotomimetic cannabinoid, currently undergoing phase 2 clinical trials for medical use in humans, has been reported to bind to TRPV1, with unknown effects on adult neurogenesis. Using the neurosphere assay, cells were subjected to pharmacological treatments for 2 or 7 days according to the experimental protocol. CBDV-treated cells for 2 days in vitro (DIV2) promoted an increase in cell survival (PI^+cells) and cell proliferation ($BrdU^+$ cells). While at DIV7, CBDV promoted an increase in neuronal differentiation ($NeuN^+$ cells) and inhibited oligodendroglial maturation (). Importantly, TRPV1 antagonist 5'-Iodoresiniferatoxin blocked the effects on cell death and cell proliferation mediated by CBDV, further suggesting TRPV1-dependency. In vivo, using a female mouse model of RTT, animals were subjected to a chronic treatment with CBDV on the pre-symptomatic stage, followed by a battery of behaviour tests, used to gauge the putative therapeutic effects of CBDV administration. The Novel Object Recognition Test suggested that CBDV ameliorates cognitive impairments in these animals. No odour disfunction, motor coordination and anxiety impairments were found in RTT animals. Locomotion deficits were not recovered by treatment with CBDV. Additionally, RTT animals show an increased abnormal hippocampal neurogenesis that tends to be attenuated in CBDV treated animals. Taken together, this project explores the novel neurogenic potential of CBDV via TRPV1. These findings will provide new insights for future research aiming to repurpose CBDV as a viable drug to treat RTT.

\ A novel ketogenic diet slows down seizure development in the rapid kindling rat model of epileptogenesis

H. Meeusen[1,2],
D.W.M. Broekaart[1], R. Kalf[1], J.P. Silva[2], J.M. Verkuijl[2], A. van Helvoort[2,3], J.A. Gorter[4], E.A. van Vliet[1,4,*], E. Aronica[1,5,*]

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[3]NUTRIM –School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Center+, Maastricht, the Netherlands
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[5]Stichting Epilepsie Instellingen Nederland, Heemstede, the Netherlands
* Shared senior authorship

Abstract:

Background: Patients with drug-resistant epilepsy need alternative therapies in order to adequately treat their recurrent seizures. Although the ketogenic diet (KD) can be an effective treatment option for these patients, the high fat content of the diet introduces tolerability and compliance challenges. To mitigate these issues, we developed a novel KD with a lower fat content and added nutrient combination for neuroprotection, optimized ketogenesis and restriction of glycolysis. The aim is to compare its efficacy to a control diet and classic KD.

Methods: The diets were initiated one week prior to testing seizure development. We used the rapid hippocampal kindling and compared seizure development using the Racine's scale and EEG afterdischarge durations. Blood ketone levels were monitored and behavioral effects of the rapid kindling paradigm and diets were tested in the open field at day 5 after the last kindling.

Results: Both novel and classic KD groups entered ketosis before the onset of rapid kindling, but ketone levels were significantly higher in the classic KD group. The novel KD significantly reduced afterdischarge duration and the rate of seizure progression. The classic KD also shortened after-discharges, but seizure progression was not affected. Rapid

kindling increased open field activity and exploration parameters and these were normalized by both KDs.

Conclusions: Inhibition of seizure progression is more effective in the novel KD group compared to the classic KD group, despite the lower ketone levels in the novel KD group than in the classic KD group. This suggests that alternative mode(s) of action could contribute to the observed effects on seizure progression. The novel KD's reduced fat content and enhanced effectiveness in this model of epileptogenesis signify a potentially promising novel therapy for epilepsy patients.

\ Peptide shuttles for receptor independent transport across the BBB

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[2]Proteomics and Protein Chemistry Unit, Department of Experimental and Health Sciences, Pompeu Fabra University, Dr. Aiguader 88, Barcelona Biomedical Research Park, 08003 Barcelona, Spain.
[3]Centro de Ciências e Tecnologias Nucleares and Departamento de Engenharia e Ciências Nucleares, Instituto Superior Técnico, Universidade de Lisboa, CTN, Estrada Nacional 10 (km 139,7), 2695-066 Bobadela LRS, Portugal.

Abstract:

The delivery of therapeutic molecules to the central nervous system (CNS) remain difficult to translate into improved clinical outcomes. This is largely due to the blood–brain barrier (BBB), the most tightly regulated interface in the human body, which can exclude most therapeutics. Therefore, for brain delivery, there is need to modify a drug to facilitate BBB crossing, e.g using endogenous mechanisms, such as receptor-mediated transport (RMT) and adsorptive-mediated transport (AMT). The focus of this presentation is the application of AMT for BBB crossing for the delivery of drugs for the treatment of brain diseases, such as pain, Alzheimer's disease and brain metastasis. We have developed a BBB peptide shuttle (BBBpS), PepH3 that presents high brain penetration in vitro and in vivo [1, 2]. PepH3 has been successfully conjugated to therapeutics peptides, proteins and antibody fragments, improving their ability to cross the BBB in vitro and in vivo [3-6].

References:

(1) Neves V et al. Novel Peptides Derived from Dengue Virus Capsid Protein Translocate Reversibly the Blood-Brain Barrier through a Receptor-Free Mechanism. ACS Chem Biol, 2017.
(2) Cavaco MC et al. DPepH3, an Improved Peptide Shuttle for Receptor-independent Transport across the Blood-Brain Barrier. Curr Pharm Des, 2020.
(3) Côte-Real et al. Antibody Molecules and Peptide Delivery Systems For Use In Alzheimer's Disease And Related Disorders. 2016, WO2016120843A1
(4) Neves-Coelho et al. A New Noncanonical Anionic Peptide That Translocates a Cellular Blood–Brain Barrier Model. Molecules, 2017.
(5) Gallo M et al. Orally Active Peptide Vector Allows Using Cannabis to Fight Pain While Avoiding Side Effects. J Med Chem. 2021 May 27;64(10):6937-6948

(6) Cavaco M et al. Conjugation of a Blood Brain Barrier Peptide Shuttle to an Fc Domain for Brain Delivery of Therapeutic Biomolecules. ACS Medicinal Chemistry Letters 2021 12 (11), 1663-1668.

\ Cytokines as potential modulators of neurotransmission in drug-resistant epilepsy: a potential beneficial effect of IL-10

Gabriele Ruffolo[1],
Alessia Romagnolo[2], Veronica Alfano[1], Till Zimmer[2], James Mills[2], Pierangelo Cifelli[3], Eleonora Aronica[2,4] & Eleonora Palma[1]

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[2] Department of (Neuro)Pathology, Amsterdam UMC, University of Amsterdam, 1105 Amsterdam, The Netherlands
[3] Department of Applied Clinical and Biotechnological Sciences, University of L'Aquila, 67100 L'Aquila, Italy
[4] Stichting Epilepsie Instellingen Nederland, 0397 Heemstede, The Netherlands

Abstract:

Drug-resistant epilepsy (DRE) is considered a daunting issue in clinical practice and new therapeutic strategies would benefit all patients belonging to this category. In this regard, an intriguing and partially unexploited field is that of cytokines and chemokines, key endogenous modulators of inflammation, which have opened promising perspectives both in preclinical and clinical research (*Noe et al., 2013; Kenney-Jung et al., 2016*). In particular, the ability of these mediators to influence neurotransmission is also worthy of further exploration. In this regard, while the role of pro-inflammatory cytokines has been well established less is known about anti-inflammatory ones, such as IL-10.

Previous studies (*Roseti et al., 2015*) determined that IL-1β can decrease $GABA_A$Rs function in temporal lobe epilepsy (TLE). Hence, we evaluated the effect of IL-10 and IL-17 on neurotransmitter receptors (Rs), such as $GABA_A$Rs and AMPARs, and analyzed the signaling pathways activated by this cytokine in DREs. Here, we performed electrophysiology experiments using tissues from patients afflicted by Tuberous Sclerosis Complex (TSC) and Ganglioglioma (GG) to determine if cytokines as IL-10 and IL-17 may modulate neurotransmission in these samples.

We found that IL-10, in contrast with IL-17, is able to increase the amplitude of GABA-evoked responses especially in tissues from GG, in line with a bioinformatics study that disclosed an upregulation of both IL-10R and its downstream signaling in these tissues. On the other hand, IL-10 has no effect on TLE tissues. These results point out that the enhancement of inhibitory neurotransmission may be a mechanism contributing to

cytokine-mediated neuroprotection, as also described previously (Roseti et al., 2013), and further experiments will better elucidate these preliminary results.

References:

Kenney-Jung DL, Vezzani A, Kahoud RJ, LaFrance-Corey RG, Ho M-L, Muskardin TW, et al. Febrile infection-related epilepsy syndrome treated with anakinra. Ann Neurol. 2016 Dec;80(6):939–45.

Noe FM, Polascheck N, Frigerio F, Bankstahl M, Ravizza T, Marchini S, et al. Pharmacological blockade of IL-1β/IL-1 receptor type 1 axis during epileptogenesis provides neuroprotection in two rat models of temporal lobe epilepsy. Neurobiol Dis. 2013 Nov;59:183–93.

Roseti C, van Vliet EA, Cifelli P, Ruffolo G, Baayen JC, Di Castro MA, et al. GABAA currents are decreased by IL-1β in epileptogenic tissue of patients with temporal lobe epilepsy: implications for ictogenesis. Neurobiol Dis. 2015 Oct;82:311–20.

\ Selective inhibition of synaptic transmission by MRS5474 in epileptic rats but not in control ones

Anwesha Ghosh [1,2],
Leonor Ribeiro-Rodrigues[1,2], Nádia Rei[5], Dilip K. Tosh[6], Tatiana P Morais[3,4], Sandra H Vaz[1,2], Cláudia A Valente,[1,2], Kenneth A Jacobson [6], Joaquim A Ribeiro[1,2], Ana M Sebastiao[1,2]

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Abstract:

Resistance to pharmacotherapy requires the development of novel antiepileptic drugs (AEDs). An adenosine A_1 receptor (A_1R) agonist, MRS5474, possesses anticonvulsant activity (Tosh et al., 2012, J Med Chem, 55:8075), without the cardiac side effects of other A_1R agonists. We hypothesized that it could operate via a novel mechanism. We thus assessed its influence upon hippocampal excitatory transmission and GABA uptake in control and in status-epilepticus (SE)-induced conditions.

Excitatory post-synaptic potentials (fEPSPs) or population spikes (PS) were recorded from the CA1 area of hippocampal slices. Status epilepticus (SE) was induced by kainate (10mg/kg, i.p) 4 weeks before hippocampal slice preparation, as approved by the ethics committee. [^{3}H] GABA uptake from rat hippocampal slices was performed as reported (Chazalon et al. Cell Rep 2018. 23:1678). GAT-1 mediated uptake was taken as the subtraction between total uptake and uptake in the presence of the GAT-1 inhibitor SKF 89976A (20μM).

In control slices MRS5474 (120-500nM) was devoid of effect in fEPSPs (n=7, mice; n=2, rat) or PS (n=2, rat). In contrast, the well-known A_1R agonists, CPA (30nM, n=2), or CHA (30nM, n=2), caused the expected marked inhibition (>60%) of fEPSPs. Remarkably, in SE rat slices, MRS5474 (250nM) clearly inhibited (45±8.9%, n=4, $p<0.05$) fEPSP slope and PS amplitude (52±15%, n=2).

In control slices, however, MRS5474 (50nM) inhibited GAT-1 mediated [^{3}H] GABA uptake by 53±10% (n=5, $p<0.05$), but this effect is likely not mediated by A_1R since it was not

blocked by the A_1R antagonist, DPCPX (50nM, % inhibition 46±6.2, n=4, $p>0.05$ as compared with absence of DPCPX).

Altogether the data shows that MRS5474 does not share properties with canonical A_1R agonists, suggesting that this putative AED may have fewer side effects than other A_1R agonists and currently available AEDs.

Project funded by Fundaç ão para a Ciê ncia e Tecnologia, Portugal (PTDC/MED-FAR/30933/2017) and by the European Union's Horizon 2020 research and innovation programme (grant agreement No. 952455, EpiEpiNet Twinning project).

\ The Neuron Hunters: a 18 years long story spent on human brain tissue

Katiuscia Martinello[1]

[1] Neurophysiology- Inflammaging unit. IRCCs Neuromed

Abstract:

Temporal lobe epilepsy (TLE), the most representative adult epilepsy, is associated with an imbalance between inhibitory and excitatory neurotransmission. A large percentage of TLE patients become drug-resistant and, if possible, undergo neurosurgery resection of the epileptic focus. Since 2004, at IRCCS Neuromed, in Pozzilli, The Experimental Epilepsy Lab people, by using the patch-clamp technique in human ex vivo tissues, has characterized several aspects linking the change of GABAergic neurotransmission to TLE physiopathological process:

1) We described for the first time a use-dependent rundown of neocortical GABA(A)-receptor which represents an epileptic-specific dysfunction which could be partially recovered by BDNF application and by acting on adenosine receptors pathway [(1-3)];

2) By patching human L5 neurons, our data also demonstrate that, in TLE tissues, the reduced GABAergic function can be modulated by CX3CL1 and that, in these tissues, we observed an increase of CX3CR1 expression in activated microglia. Therefore, we linked an epilepsy hallmark (IGABA rundown) to the inflammatory process disclosing a potential role of microglia in the propagation of seizures [(4)];

3) At synaptic level, we illustrated constitutive functional crosstalk between GABAB and GABAA receptors in human temporal layer 5 pyramidal neurons, which is lost in epileptic tissues. In seizure-free human tissues, the activation of GABAB receptors produced the increase of the inhibitory net charge associated with a single synaptic event involving the activation of Protein kinase A (PKA) in the postsynaptic cells. This crosstalk disappeared in TLE tissue, reducing the effect of GABAergic synaptic neurotransmission[(5)];

4) We have characterized the excitability of both interneurons and pyramidal cells at CA3 in sclerotic hippocampi associated or not with a familiar SCN1A mutation [(6)];

5) We have studied the role of the cholinergic system in modulating inhibitory and excitatory neurotransmission epileptic tissues by using neuronal nicotinic receptors positive allosteric modulators (Martinello et al, under revision).

References:

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\ The puzzle: absence seizures, astrocyte complexity and memory impairments

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Abstract:

Typical absence seizures (ASs), characterized by 3-4 Hz spike-wave discharges in thalamocortical network, are the hallmark of Childhood Absence Epilepsy (CAE). In ASs animal models, dysfunction of thalamic GABA transporter-1 (GAT-1), exclusively expressed in thalamic astrocytes and responsible for GABA reuptake, enhances tonic inhibition. This enhancement is essential and sufficient for the generation of ASs. A neuropsychological impairment is observed in 60% of children with CAE. Using an ASs animal model, the Genetic Absence Epilepsy Rat from Strasbourg (GAERS), the respective Non-Epileptic control (NEC), and Wistar rats, behavior tests were carried out to assess learning and memory. Hippocampal synaptic plasticity was evaluated by performing long-term potentiation experiments where field excitatory postsynaptic potentials were recorded. Immunohistochemistry and Western blot assays were used to assess molecular and cellular morphological differences between epileptic and non-epileptic animals. In Novel object recognition, Cross-modal object recognition, Y-Maze and Barnes Maze tests, we observed significant differences between GAERS and controls, suggesting an impairment in the prefrontal cortex, parahippocampal regions and hippocampal-dependent memory. LTP magnitude in GAERS was significantly lower than from Wistar. GAERS display an increase in astrocytic morphological complexity, mainly in hippocampus, motor cortex and somatosensory cortex. GAERS also display increase GFAP expression levels, compared to Wistar and NEC in all brain regions tested, except in the somatosensory cortex. Our results suggest memory deficits in GAERS, especially in spatial

working memory, object recognition and long-term memory, in line with the reduced LTP magnitude. Since GAT-1 is expressed in astrocytes and there is an increase in morphological complexity and GFAP expression in GAERS, the involvement of astrocytes in the pathology of ASs is reinforced.

Acknowledgements: Authors declare no conflict of interests. This project was funded by FCT (PTDC/BTM-SAL/32147/2017).

The relevance of Mitochondrial Dynamics in Neural Stem Cell Fate

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Abstract:

Neural stem cells (NSCs) are found in discrete regions of the adult mammalian brain[1]. During adulthood, NSCs can differentiate into neurons, astrocytes and oligodendrocytes, making them a powerful tool to treat disease-related neural loss. Several studies suggest that mitochondria have an important role in regulating NSC differentiation and lineage determination[2]. A major aspect that remains unclear is whether mitochondrial dynamics have a role in directing NSC fate. Hence, our work aims to dissect how mitochondria biogenesis, morphology and bioenergetics can modulate NSC differentiation. For this, NSCs were obtained by isolating subventricular zone (SVZ) and dentate gyrus (DG) cells from P1-3 C57Bl6 mice[3]. The isolated cells were grown in neurospheres, and consequently passaged to guarantee higher yields of NSCs. Thereafter, neurospheres were plated under specific differentiation conditions giving raise to neurons, astrocytes and oligodendrocytes. Additionally, expression of proteins involved in mitochondrial biogenesis and fusion/fission was determined. Overall, expression of mitochondrial biogenesis-related proteins did not significantly change with NSC differentiation, in both neurogenic niches. Importantly, the levels of proteins involved in mitochondrial fusion (Mfn1/Mfn2) significantly increased while proteins involved in fission (DRP1) significantly decreased along differentiation, only in SVZ cells. Furthermore, mitochondrial number, length and area was different in the different cell types (NSCs and differentiated cells). Indeed, mitochondrial number significantly increased during astroglial and neuronal differentiation. Moreover, both NSCs and oligodendrocyte precursor cells were the cells with more elongated mitochondria. Interestingly, mitochondrial area did not change in neuronal cells, while there were significant alterations along oligodendroglial

differentiation. These results will pave the road towards novel findings concerning the role of mitochondrial dynamics in NSC fate.

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SYNAPTIC DYSFUNCTIONS & EXCITABILITY IN EPILEPSIES

\ POSTER 1 – FXS-patient derived cortical organoids integrating microglia as 3D model system to dissect the neurodevelopmental roots of the disease.

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Abstract:

Fragile X syndrome (FXS) is the most common inherited form of human mental retardation, and it is caused by expansion of CGG repeat in the FMR1 gene. The resulting epigenetic silencing causes the loss of the fragile X mental retardation protein (FMRP) with defects in the regulation of dendritic spine morphology and synaptogenesis. FXS is widely studied into 2D cell culture differentiating human induced pluripotent stem cells (iPSCs) into neuronal population to characterize the disease phenotype taking advantages of molecular and functional analysis. However, conventional 2D cell culture fails to recapitulate the complex neural environment revealing itself as a not reliable in vitro model system to fully characterize the pathology. In this direction novel 2D and 3D model systems have been proposed for dissecting the molecular and cellular processes underling FXS. Several 3D protocols are available to better mimicking the cell complexity and architecture of the brain tissue, however the lack of non- neural cell types such as microglia still hinders their exploitation for the study of the neuro-immune axis in neurodevelopmental diseases. The aim of our study is to create an in vitro 3D model based on patient-specific induced pluripotent stem cells (iPSCs) with the purpose of deciphering the neurobiological phenotypes associated with FXS. Specifically, we propose to co-culture iPSC-derived cortical organoids and isogenic iPSC-derived microglia to generate a disease-relevant and tailored platform for the investigation of neuro-immune interaction during brain development. Indeed, microglia plays a prominent role in shaping synaptic circuitries during neurodevelopment and its presence might unveil possible neural-immune interplay at the basis of FXS and the establishment of a mature synaptic transmission.

\ POSTER 2 – The neurogenic potential of caffeine: an *in vitro* study using the neurosphere assay

João B. Moreira[1,2], Marit A. Vink[1,2], Rui S. Rodrigues[1,2], Ana M. Sebastião[1,2], Sabine Lévi[3,4,5], Luísa V. Lopes[2], Sara Xapelli[1,2]

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Abstract:

Neurogenesis, the formation of neurons through the differentiation of neural stem cells (NSCs), occurs in the adult mammalian brain, primarily in the subventricular zone (SVZ) and dentate gyrus (DG) of the hippocampus. Caffeine, the most widely used psychostimulant, acts as a potent non-selective adenosine receptor antagonist. Although some studies suggest that caffeine influences NSCs proliferation in a time and dose-dependent manner, its comprehensive impact on the regulation of brain plasticity has been largely overlooked. Thus, the goal of our project is to dissect caffeine effects on proliferation and differentiation of adult NSCs.

The effects mediated by caffeine in different neurogenic features was evaluated *in vitro*, using SVZ and DG neurospheres from P1-3 C57Bl6 mice that were pharmacological treated with several concentrations of caffeine (10, 80, 125, 250 µM and 1mM). Cell proliferation was analyzed by BrdU staining, cell survival by propidium iodide staining, and neuronal differentiation by BrdU/NeuN staining.

No significant alterations in cell survival (PI+ cells) at DIV1 were observed in cultures derived from both neurogenic niches (n=4). Preliminary data suggests that caffeine 125µM promoted whilst 1mM decreased cell proliferation (BrdU+ cells) at DIV1 in SVZ (n=7-8). Moreover, caffeine (1 mM) significantly increased the number of mature neurons (NeuN+ cells) at DIV7 in SVZ-derived neurospheres (n=5, $p< 0.0001$).

This project will unveil the mechanisms underlying caffeine effects in neurogenesis. Importantly, novel insights about the caffeine effects in brain physiology will be discovered which will have a relevant impact in public health.

\ POSTER 3 – Postnatal oligodendrogenesis is potentiated by BDND *in vitro*

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Abstract:

Oligodendrocytes (OLs) are the myelin-forming cells in the Central Nervous System of vertebrates. Under demyelinating conditions, oligodendrocyte precursor cells (OPCs) present in the brain parenchyma or derived from subventricular zone neural stem cells (SVZ-NSCs) can differentiate into oligodendrocytes (OLs), which migrate and partially remyelinate the lesioned areas. The role of modulators such as brain-derived neurotrophic factor (BDNF) and adenosine A2A receptors (A2ARs) on adult oligodendrogenesis from SVZ-NSCs remains unknown.

Hence, we aimed at studying how these modulators and the putative crosstalk between BDNF and A2ARs can influence OL differentiation from postnatal SVZ-NSCs.

Results obtained using SVZ-NSCs that were pharmacologically treated with BDNF (30 ng/mL), A2AR agonist (CGS21680, 30 nM) and A2AR antagonist (ZM 241385, 50 nM) show that treatment with BDNF tends to increase OPC formation (NG2/PDGFRα-positive cells) after 4 days in vitro (DIV) (n=3; CTRL set to 100%, BDNF 203.8±27.59; p=0.0548), whilst significantly increasing the number of OPCs at DIV7 (n=7-8; CTRL set to 100%, BDNF 210.2±21.87; p<0.0001) without affecting OL maturation (MBP-positive cells). Importantly, BDNF effects on OPC formation at DIV7 were partially abrogated by the A2AR antagonist (n=4-8; CTRL set to 100%, BDNF+ZM 174.0±8.951; p<0.01), while the antagonist by itself had no effect when comparing with control (ZM 117.6±15.47; p>0.05). However, no changes were observed after treatment with the A2AR agonist at these timepoints in both OPC formation and OL maturation.

To date, this work outlined the role of BDNF in promoting the formation of OPCs derived from SVZ-NSCs. We are currently addressing if the effect of BDNF is dependent of A2ARs throughout OL maturation and also during proliferation (through BrdU staining protocol). Ultimately our work will contribute to the development of alternative therapeutic targets for OL formation and remyelination.

Funding:

Authors received funding from European Union's Horizon 2020 research and innovation programme under the grant agreement No. 952455, Fundação para Ciência e Tecnologia with the project IF/01227/2015 (for SX), the fellowships PD/BD/150343/2019 (for JMM) and PD/BD/141784/2018 (for DML) and the GAPIC project 20210009. The authors declare no conflicts of interests.

\ POSTER 4 - The puzzle: absence seizures, astrocyte complexity and memory impairments

Mariana Neuparth Sottomayor[1,2], Carolina C. Pina[1,2], Miguel Farinha-Ferreira[1,2], Filipa Solano[1,2] Daniela Abreu[1,2], Francisco Mouro[1,2], Tatiana P. Morais[3,4], Ana Maria Sebastião[1,2], Vincenzo Crunelli[3,4], Sandra H. Vaz[1,2]

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Abstract:

Typical absence seizures (ASs), characterized by 3-4 Hz spike-wave discharges in thalamocortical network, are the hallmark of Childhood Absence Epilepsy (CAE). In ASs animal models, dysfunction of thalamic GABA transporter-1 (GAT-1), exclusively expressed in thalamic astrocytes and responsible for GABA reuptake, enhances tonic inhibition. This enhancement is essential and sufficient for the generation of ASs. A neuropsychological impairment is observed in 60% of children with CAE. Using an ASs animal model, the Genetic Absence Epilepsy Rat from Strasbourg (GAERS), the respective Non-Epileptic control (NEC), and Wistar rats, behavior tests were carried out to assess learning and memory. Hippocampal synaptic plasticity was evaluated by performing long-term potentiation experiments where field excitatory postsynaptic potentials were recorded. Immunohistochemistry and Western blot assays were used to assess molecular and cellular morphological differences between epileptic and non-epileptic animals. In Novel object recognition, Cross-modal object recognition, Y-Maze and Barnes Maze tests, we observed significant differences between GAERS and controls, suggesting an impairment in the prefrontal cortex, parahippocampal regions and hippocampal-dependent memory. LTP magnitude in GAERS was significantly lower than from Wistar. GAERS display an increase in astrocytic morphological complexity, mainly in hippocampus, motor cortex and somatosensory cortex. GAERS also display increase GFAP expression levels, compared to Wistar and NEC in all brain regions tested, except in the somatosensory cortex. Our results suggest memory deficits in GAERS, especially in spatial

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Acknowledgements: Authors declare no conflict of interests. This project was funded by FCT (PTDC/BTM-SAL/32147/2017).

NEUROINFLAMMATION & GLIAL CELLS IN EPILEPSIES

\ POSTER 5 – Functional characterization of iPSC-derived human neurons from neurological patients

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Abstract:

In the last few years, the use of human iPSCs (induced Pluripotent Stem Cell)-derived cells is increasingly acquiring importance. It is of great importance to enlarge the use of this cell biology technique in several pathologies affecting cells not easily available for experimental activity, such as neurons. With this approach, the cellular and molecular research on different neuropathologies, such as epilepsy and neurodegenerative diseases, will be simpler and more productive.

In this perspective, we are characterizing the functional properties of different human iPSC-derived neuronal subtypes, including spinal motor neurons and cortical GABAergic interneurons. Employing electrophysiological techniques (patch-clamp) and digital time-resolved fluorescence microscopy, we aim to highlight the differences between controls and patients affected by different pathologies: ALS related to mutant FUS, and epilepsy due to a mutated voltage-gated Na+ channel.

\ POSTER 6 – Enriched Environment Cues Suggest a New Strategy to Counteract Glioma: Engineered rAAV2-IL-15 Microglia Modulate the Tumor Microenvironment

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Abstract:

Several types of cancer grow differently depending on the environmental stimuli they receive. In glioma, exposure to an enriched environment (EE) increases the overall survival rate of tumor-bearing mice, acting on the cells that participate to define the tumor microenvironment. In particular, environmental cues increase the microglial production of interleukin (IL)-15 which promotes a pro-inflammatory (antitumor) phenotype of microglia and the cytotoxic activity of natural killer (NK) cells, counteracting glioma growth, thus representing a virtuous mechanism of interaction between NK cells and microglia. To mimic the effect of EE on glioma, we investigated the potential of creating engineered microglia as the source of IL-15 in glioma. We demonstrated that microglia modified with recombinant adeno-associated virus serotype 2 (rAAV2) carrying IL-15 (rAAV2-IL-15), to force the production of IL-15, are able to increase the NK cells viability in coculture. Furthermore, the intranasal delivery of rAAV2-IL-15 microglia triggered the interplay with NK cells in vivo, enhancing NK cell recruitment and pro-inflammatory microglial phenotype in tumor mass of glioma-bearing mice, and ultimately counteracted tumor growth. This approach has a high potential for clinical translatability, highlighting the therapeutic efficacy of forced IL-15 production in microglia: the delivery of engineered rAAV2-IL-15 microglia to boost the immune response paves the way to design a new perspective therapy for glioma patients.

\ POSTER 7 – Microglia differently modulate synaptic plasticity in dorsal and ventral hippocampus

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Abstract:

The hippocampus is a functionally heterogeneous structure that displays along its longitudinal axis differences in connectivity with cortical and subcortical areas, gene expression profile, neurotransmitters receptors expression, electrophysiological and morphological properties of hippocampal CA1 pyramidal neurons.
The nervous and immune systems are involved in continuous bidirectional communication. Microglial cells play important roles in regulating neuronal functions in all brain areas in both physiological and inflammatory conditions, but the function of these cells along the hippocampal axis remains unclear.
We examined the possible contribution of microglial cells in modulating short- and long-term plasticity in the CA1 region of dorsal and ventral hippocampus. To this purpose, we interfered with physiological functions of microglial cells, using different approaches: I) a pharmacological inhibition with the tetracycline antibiotic minocycline *in vitro*, II) a pharmacological depletion with PLX5622 *in vivo*, and III) a mice model of genetic microglial deletion for CX3CR1 (fractalkine receptor) in which the microglia-neuron communication, mediated by CX3CR1 and its unique neuronal ligand CX3CL1 (fractalkine), is interrupted.
We first confirm that basal level of short- and long-term plasticity (LTP) differ in the two hippocampal poles, being the LTP enhanced in the dorsal region. Interestingly, interfering with microglia, both *in vitro* (with minocycline) and *in vivo* (PLX5622 treatment), reduced dorsal LTP while increased the ventral one. This effect was recapitulated in CX3CR1 knockout mice, indicating the relevance of this signal in setting the basal level of plasticity along the hippocampal longitudinal axis.
Analysis of microglial distribution and morphology in the two hippocampal poles also shows significant differences, being the microglia less dense but with a larger cell body in

the ventral pole, supporting our hypothesis that microglia could differently modulate neuronal functions in the dorsal and ventral hippocampus.

\ POSTER 8 – SH-SY5Y-derived extracellular vesicles drive the release of a Brain-derived Neurotrophic Factor receptor fragment: relevance for Alzheimer's disease

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Abstract:

Introduction: In Alzheimer's disease (AD), the neuroprotective BDNF/TrkB-FL system is impaired due to an amyloid-beta-mediated TrkB-FL receptor cleavage and subsequent formation of an intracellular fragment (TrkB-ICD). TrkB-ICD retains its kinase activity, promotes cognitive impairments, and modifies gene expression, thus contributing to intracellular toxicity. Interestingly, AD pathological features may be disseminated by small (sEVs) or large (lEVs) extracellular vesicles, but also by soluble factors on the secretome, causing cell-to-cell toxicity. Importantly, previous studies have already detected TrkB-ICD in cerebrospinal fluid of humans, raising the possibility of its secretion. Accordingly, this work aims to assess TrkB-ICD presence in EVs and secretome.

Methods: EVs were isolated from media of control (non-transduced, CTR), GFP- and TrkB-ICD-V5-transduced (ICD-V5) differentiated SH-SY5Y cells. After purification, lEVs and sEVs were characterized regarding their morphology, size, and protein cargo by transmission electron microscopy (TEM), dynamic light scattering (DLS) and western-blot (WB), respectively.

Results: TEM and DLS analysis showed that isolated EVs exhibited a cup-shaped morphology and expected size profile for both lEVs and sEVs. WB experiments confirmed the presence of typical EV protein markers. Interestingly, we were able not only to confirm the existence of ICD-V5 in both EV types, but also the presence of the endogenous TrkB-

ICD fragment. Additionally, this fragment was also detected in the EV-depleted cell secretome.

Conclusions: Altogether, both EV populations were correctly isolated and the detection of TrkB-ICD in both lEVs and sEVs, and in the secretome soluble fraction, strongly suggest the paracrine dissemination of this fragment from cell-to-cell, propagating its putative toxicity.

Funding

This project was funded by Santa Casa da Misericórdia de Lisboa (MB37-2017), Fundação para a Ciência e a Tecnologia (FCT – PD/BD/114441/2016 PTDC/MED-NEU/31395/2017, LISBOA-01-0145-FEDER-031395, UIDB/UIDP/04138/2020, UID/DTP/04138/2019 and SFRH/BD/128738/2017) and European Union's Horizon 2020 research and innovation programme under the grant agreement No. 952455. Authors declare no conflict of interests.

\ POSTER 9 – PLX-3397 efficiently inhibits microglia in primary astrocytic cultures

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Abstract:

Astrocytes, together with microglia, constitute the brain's immune system. Astrocytes are the most abundant cells in the CNS parenchyma, that play an essential role in several brain functions, such as fine-tuning of synaptic transmission, glutamate uptake and modulation of immune and inflammatory responses, among others [1,2].
Much of the knowledge on the biology and function of astrocytes came from the study of rodent primary astrocytic cultures, since they are a convenient and easy method to settle in laboratory. The basis of the method is the dissociation of cells from the dissected brain cortex and its plating on dishes [3]. The final culture is a mixed population of 90-95% of astrocytes and a small proportion of microglia [4]. However, in a disease context, if one wants to understand the behavior of astrocytes in respect to a certain noxious stimulus, it is crucial to have a pure culture of astrocytes. If present, microglia sense the stimulus, rapidly proliferate and react towards it, making unfeasible to address the contribution of astrocytes alone.
One promising way of eliminating microglia is through the usage of a colony-stimulating factor-1 receptor (CSF-1R) inhibitor [5]. CSF-1R is a receptor that, in the CNS, is only expressed by microglia [6] and that is essential for proliferation, maturation, survival and development of these cells [7,8]. PLX-3397 is a recently described CFS-1R inhibitor that can cross the BBB and rapidly eliminate microglia[7].
As primary astrocytic cultures are the most basic, common, and useful method used to address astrocytes' function in brain diseases, this work aimed to evaluate the efficiency of PLX-3397 in depleting microglia from these cultures, in combination with the basic method of overnight shaking. Our results point to a pronounced efficacy of PLX-3397, at 1 µM, in eliminating microglia from primary astrocytic cultures without affecting astrocyte's viability and reactivity.

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\ POSTER 10 – Searching for a potential novel biomarker for Alzheimer's disease based on BDNF receptor cleavage

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Abstract:

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder, characterized by the accumulation of Amyloid Beta (Aβ) peptide **[1]**. In AD, the signaling mediated by Brain-Derived Neurotrophic Factor (BDNF) is impaired, affecting neuronal growth, differentiation, and survival **[2]**. Recently, we found that Aβ induces BDNF's receptor (TrkB-FL) cleavage, due to calpain overactivation, generating an intracellular fragment (TrkB-ICD) **[3,4]**. Furthermore, in *post-mortem* brain samples of AD patients, we found a decrease in TrkB-FL levels concomitantly with a rise in TrkB-ICD levels with disease progression **[5]**.

Considering that TrkB-ICD might be released to the cerebrospinal fluid (CSF), the aim of this project included 1) evaluation of TrkB-FL cleavage in human CSF from patients diagnosed with Mild Cognitive Impairment (MCI) due to AD (criteria of the National Institute on Aging-Alzheimer's Association, 2011) **[6]** as compared to controls (MCI patients without any markers of amyloid pathology or neurodegeneration) and 2) to perform a correlational study between TrkB-ICD levels and age, BDNF, $A\beta_{1-42}$, phosphorylated-tau, total-tau levels.

Results show that TrkB-FL and TrkB-ICD proteins are present in human CSF. Importantly, data revealed a significant increase in TrkB-ICD levels in the AD group, when compared with non-AD group. Moreover, TrkB-ICD levels in MCI due to AD patients negatively correlate with $A\beta_{1-42}$, however, they did not correlate with phosphorylated-tau and total-tau levels, suggesting that TrkB-ICD reflects the amyloid pathology rather than non-specific neuronal death.

This preliminary data opens the possibility of studying TrkB-ICD, in the future, as a biomarker for AD.

Human samples were collected within the study *entitled Beyond Beta-Amyloid - Deciphering Early Pathogenic Changes in Alzheimer's disease* (PTDC/MED-NEU/27946/2017, approved by the Ethics Committee of CAML 345/18).

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\ POSTER 11 – Is neuroinflammation present in Duchenne Muscular Dystrophy?

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Abstract:

Duchenne muscular dystrophy (DMD) is caused by mutations in the dystrophin gene, the largest one in the human genome. The absence of dystrophin causes damage to skeletal and cardiac muscle, inducing the well-known symptoms of the pathology. However, the gene is expressed also in brain and neuropsychiatric and cognitive problems, including a incidence of epilepsy higher than in the general population, are observed in DMD patients. Other comorbidities include autism, stress and depression, diseases associated with brain inflammation and glial alterations in nondystrophic people. Knowledge about neuroinflammation in DMD and in mdx mice, the typical dystrophic animal model, is extremely limited. Since neuroinflammation typically affects the phenotype of astrocytes and microglia cells, we are characterizing these cells in mdx mice, using different technical approaches Moreover, given that synaptic transmission and animal behaviour, are altered by brain inflammation, we are undertaking a study on these topics too.

\ POSTER 12 - CX3CL1 chemokine modulates synaptic function by recruiting microglial processes

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Abstract:

Microglia are the resident immune cells of the CNS. Recent studies have highlighted the role of microglia in regulating circuit formation and homeostasis. As its function is strictly related to their ability to communicate with neurons, the alteration of microglia-neurons crosstalk leads to deficits in synaptic transmission and behaviour. Among the soluble factors involved in microglia-neuron interaction, the neuronal chemokine CX3CL1 binds to its receptor CX3CR1, exclusively found on microglia, thereby regulating microglial function. Previous studies reported that CX3CL1 expression is up-regulated in the temporal neocortex of epileptic patients and epilepsy animal models. Moreover, communication mediated by the CX3CL1/CX3CR1 axis is able to monitor and alter synaptic activity under epileptic conditions. Therefore, it is critical to understand how CX3CL1/CX3CR1 crosstalk controls synaptic activity to identify the pathophysiological mechanisms of epilepsy and new approaches to ameliorate epilepsy dysfunction.

We investigated how CX3CL1/CX3CR1 signalling influence the interaction between microglia and neurons. The Cx3cr1::CreERT2;Rosa26-CAG::LSL-tomato;Thy1::GFP mouse model allowed visualization of neurons and microglia, and the conditional knockout of CX3CR1. We studied the distribution of microglia-neuron contacts in Cx3cr1 cKO and Cx3cr1 heterozygous counterparts. We found a strong decrease in density of microglia processes contacting neurons in Cx3cr1 cKO mice.

By fluorescence monitoring of microglia in Cx3cr1 heterozygous mice, we observed that CX3CL1 attracts microglial processes. These results suggest that CX3CL1/CX3CR1 axis is needed for the establishment of direct microglia-neuron interaction. Moreover, we observed that acute application

of CX3CL1 in hippocampal slices induces a reduction of AMPA receptor-mediated EPSC. Consistently, we observed a decrease in hippocampal connectivity and changes in synaptic properties and plasticity in Cx3cl1 KO mice, suggesting that CX3CL1 is a direct mediator of glutamatergic synaptic transmission.

We conclude that CX3CL1 represents a pivotal signal in microglia interaction with synapses and that this chemokine modulates synaptic function likely by attracting microglial processes.

\ POSTER 13 - Studying epilepsy at IRCSS NEUROMED: A multimethod approach for a multidomain disease

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Abstract:

Epilepsy is one of the most common chronic neurological diseases affecting around 50 million people worldwide, with temporal lobe epilepsy (TLE) being the most common type (1).

TLE is characterized by the occurrence of spontaneous seizures as a result of neuronal excitation/inhibition imbalance. Recent studies reveal that microglia also participate in the maintenance of excitation/inhibition homeostasis in line with growing evidence implicating a role of neuroinflammatory processes in the pathogenesis of epilepsy (2,3).

Moreover, deficits of cognitive and emotional processes often occur in epileptic patients with detrimental impact on social, academic and work performance, as well as quality of life (4,5).

Thus epilepsy could be defined as a brain network disease that require a multiple methods strategy of investigation.

At the Experimental Epilepsy lab - IRCSS NEUROMED we are investigating TLE at both micro and macroscopic level. In particular, we implemented the kainic acid (KA) model of TLE by intra-hippocampal infusion of KA in mice. By taking advantage of a multitechnique approach we will: i) functionally characterize epileptic neurons ex vivo (e.g. electrophysiology, neurotransmitter release manipulation); ii) correlate epileptic neuron features to behavioral phenotype by performing behavioral tasks highlighting cognitive and emotional domains typically impaired in epilepsy (e.g. recognition memory, spatial memory, emotional memory); iii) investigate features of microglia cells both ex vivo and in vitro.

This integrative research approach will allow us a better understanding of pathophysiological mechanisms of epilepsy in order to identify new therapeutic targets.

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\ POSTER 14 – Astrocytes: a model to study synaptic plasticity, learning and memory, and disease research

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Abstract:

Introduction: The dn-SNARE mouse model is being used as a tool to dissect the role of gliotransmitter release in the function of brain circuits and consequent behavior modulation. These mice have exocytosis selectively impaired in astrocytes via conditional expression of a dominant-negative SNARE protein under the control of the GFAP promoter (1). This model has already been proved to be effective in the study of epilepsy since in dn-SNARE mice there is an amelioration of the progressive increase in seizure frequency, hippocampal sclerosis, and behavioral abnormalities (2). Moreover, despite being a widely used model, the effectiveness of dn-SNARE mouse model as a good model to study SNARE-dependent vesicular release of astrocytic gliotransmitters was questioned (3). Although efforts have been made to produce evidence to support this model (4), we performed complementary experiments to revalidate the dn-SNARE mouse model, and thus highlight astrocytes' contribution to neurotransmission through SNARE-dependent release of gliotransmitters.

Materials and Methods: Immunohistochemical was performed to confirm dn-SNARE GFP reporter transgene expression is astrocyte-specific. Field excitatory postsynaptic potentials (fEPSPs) were recorded from the CA1 area of hippocampal slices from dn-SNARE and Wt male mice (8-12 weeks). To study synaptic plasticity, LTP was induced by theta-burst stimulation, Input/Output curves were recorded to assess synaptic efficiency and paired-pulse facilitation protocol was induced to analyze pre-synaptic activity. In addition, to evaluate the role of astrocyte-derived gliotransmitters in cognitive function, both groups of animals were submitted to different behavior tests at 20 weeks, including the open field test (OFT) to assay general locomotor activity levels, anxiety, and willingness to explore, Y-maze spontaneous alternation for measuring spatial working memory, novel

object recognition (NOR) to assess memory and discrimination, and Morris water maze (MWM) to test spatial memory and long term memory.

Results: Immunohistochemical analysis confirmed that GFP reporter transgene co-localizes with GFAP-positive astrocytes of dn-SNARE mice and is absent in Wt mice hippocampus. Additionally, GFP failed to show co-localization with βIII-tubulin (axonal marker) of dn-SNARE animals, supporting astrocyte-specific dn-SNARE expression and excluding the potential neuronal expression. Blocking astrocytic gliotransmission decreases LTP and basal synaptic transmission, without interfering with presynaptic mechanisms associated with the release of neurotransmitters to the synapse. Furthermore, behavioral preliminary results suggest that the impairment of the exocytotic release of gliotransmitters by astrocytes, in OFT, is not sufficient to cause an alteration in the locomotor activity but may influence exploratory behaviors and anxiety patterns. In the Y-maze test, blockade of gliotransmission does not affect spatial working memory or the function of the prefrontal cortex. Moreover, in the NOR, gliotransmission impairment in astrocytes does interfere with the ability to recognize changes in a novel object in an otherwise familiar environment. Lastly, in MWM, animals with compromised gliotransmission traveled longer distances and spent less time in the correct (on which the platform was previously held), suggesting that astrocytes modulate spatial and long-term memory.

Discussion/Conclusion: Our data suggest cellular specificity of the dn-SNARE transgene expression in dn-SNARE mice and extend prior reports documenting the validity and relevance of a model to study astrocytic gliotransmission. Therefore, this model is an excellent tool to study the impact of astrocyte signaling on the modulation of synaptic plasticity and learning and memory processes.

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NOVEL THERAPIES FOR EPILEPSY TREATMENT & DEVELOPMENTAL EPILEPSIES

\ POSTER 16 – Exploration of miRNAs expression pattern in mTORopathies

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Abstract:
Malformations of cortical development (MCDs) are congenital lesions responsible for epilepsy and developmental delay through multiple genetic etiologies, anatomic and cellular abnormalities. Secondary to these cortical lesions in the brain, episodes of focal epilepsy occur causing the formation of epileptogenic lesions. Genetic alterations in the mTOR pathway may lead to the development of Focal Cortical Dysplasia type 2a and type 2b (FCD 2a and 2b), and Tuberous Sclerosis Complex (TSC), which are known to be the most common causes of refractory epilepsy in the pediatric population.
Using high-throughput RNA sequencing (RNA-seq) and small RNA-seq, a comprehensive overview of the protein-coding and non-coding transcriptome of the brain was created. In the context of small noncoding RNAs, microRNAs (miRNAs) have gained traction due to their impact on the protein-coding transcriptome and, therefore, their impact on the disease pathology. In the present study, the diseaserelated miRNA expression in epileptogenic lesions was investigated. We observed a strong correlation between miRNAs expression and age in the control cortex. In contrast, the disease process involved in mTORopathies may disrupt the developmental expression patterns of miRNAs in the resected lesions. The differential expression analysis revealed 66 common miRNAs across the three pathologies. Furthermore, the number of overlapping differentially expressed miRNAs between FCD 2a-FCD 2b, FCD 2b-TSC and FCD 2a-TSC were compared, showing closer expression profile of FCD 2b to TSC. Three miRNAs of interest were selected for further investigation regarding their role in the neurobiology of epileptogenic lesions, further this study also provides a comprehensive overview of the non-coding transcriptional landscape of mTORopathies.

\ POSTER 17 – TrkB-FL cleavage and anxiety-like behaviour is dependent on seizure severity in a kainate-induced SE model

Leonor Ribeiro-Rodrigues[1,2], João Fonseca-Gomes[1,2], Sara L. Paulo[1,2], Svitlana Zavalko[1,2], Vitor H. Paiva[3], Ana M. Sebastião[1,2], Maria José Diógenes[*,1,2], Sara Xapelli[*,1,2]

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*similar contribution

Abstract:

In epilepsy, changes in BDNF levels have been described, while in excitotoxic conditions, TrkB-FL receptor is cleaved, forming an intracellular fragment: TrkB-ICD. In an in vitro model of status epilepticus (SE), this cleavage was also suggested. Therefore, the aim of this work was to study whether the cleavage of TrkB-FL occurs in an in vivo model of SE and chronic epilepsy and if it is related to seizure severity and behaviour alterations.

A model of epilepsy was induced with kainate (KA, ip) in rats. Two groups of animals were obtained: SE group sacrificed 1.5-4 hours after KA; and chronic group which had spontaneous seizures 4 weeks after KA. The modified Racine Scale was used to classify the seizures severity.

In the SE group, TrkB-FL protein levels were significantly decreased in animals with the highest seizure score. Importantly, there was a positive significant effect of TrkB-ICD levels on the number of seizures, suggesting that animals with more seizures have higher levels of TrkB-ICD.

In the chronic group, there was no evidence of TrkB-FL cleavage with or without spontaneous seizures. Preliminary behaviour characterization showed that animals displaying spontaneous seizures had a less anxious-like behaviour and an increase in locomotor activity.

Taken together, our results show that animals with the highest seizure score severity in SE have a degree of TrkB-FL cleavage directly proportional to the number of seizures. However, the TrkB-FL cleavage may not be continuously occurring during the process of epileptogenesis. Intriguingly, data suggest that animals with chronic epilepsy have less anxious-like behaviour.

\ POSTER 18 – Establishing a new method for TrkB-FL cleavage detection in murine CSF and plasma

Nuno Alemãn-Serrano [1,2]*, Rita Cardoso do Amaral [1,2]*, Carolina de Almeida-Borlido [1,2], Tiago Costa-Coelho [1,2], Catarina Miranda-Lourenço [1,2], Leonor Ribeiro-Rodrigues [1,2], Ana. M. Sebastião [1,2], Maria José Diógenes [1,2]

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* Equal Contribution

Abstract:

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the accumulation of amyloid beta (Aβ) peptide. In AD, the signaling mediated by Brain-Derived Neurotrophic Factor (BDNF) is impaired, affecting neuronal growth, differentiation, and survival.
Recently, we found that Aβ induces BDNF receptor (TrkB-FL) cleavage, due to calpain overactivation, generating an intracellular fragment (TrkB-ICD).
Supporting this evidence, in *post-mortem* brain samples of AD patients, we found decreased TrkB-FL levels together with an increase in TrkB-ICD levels, in later stages of the disease. Furthermore, recent studies have shown that TrkB-ICD levels are increased in the Cerebrospinal Fluid (CSF) of AD patients when compared to same aged control groups.
To counteract TrkB-FL loss, we developed a new compound able to prevent TrkB-FL cleavage, TAT-TrkB, that has already shown promising results. Therefore, the aim of this project is to optimize the methodology to evaluate TrkB-ICD levels in both CSF and plasma from mice and to evaluate whether these may be used to monitor the effect of TAT-TrkB and AD progression.
A total of 10-15 µl of CSF per mice was collected from the cisterna magna by glass capillary puncture. CSF was stored at -80ºC after a 2000g centrifugation for 2 min at RT. Blood was collected upon decapitation and centrifuged (2000g). The obtained plasma was stored at -80ºC. Western blot analysis of 15 µg of CSF and plasma proteins allowed the detection of TrkB-ICD using the Pan-Trk antibody.

The performed experiments allowed us to find the proper conditions to detect TrkB-ICD by western blot.

Funding:

Authors received funding from European Union's Horizon 2020 research and innovation programme under the grant agreement No. 952455; 24º "Educação pela Ciência" Programme by GAPIC-Gabinete de Apoio à Investigação Científica, Tecnológica e Inovação (Project 20210028); Santa Casa da Misericórdia de Lisboa (MB37-2017); Fundação para a Ciência e Tecnologia (FCT –(PD/BD/150344/2019); PTDC/MED-NEU/27946/2017).

\ POSTER 19 – The relevance of Mitochondrial Dynamics in Neural Stem Cell Fate

Rita Soares[1,2,3], Isa F. Mota[1,2,3], Diogo M. Lourenço[1,2], Ana M. Sebastião[1,2], Sara Xapelli[1,2], Vanessa A. Morais[1,3]

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Abstract:

Neural stem cells (NSCs) are found in discrete regions of the adult mammalian brain[1]. During adulthood, NSCs can differentiate into neurons, astrocytes and oligodendrocytes, making them a powerful tool to treat disease-related neural loss. Several studies suggest that mitochondria have an important role in regulating NSC differentiation and lineage determination[2]. A major aspect that remains unclear is whether mitochondrial dynamics have a role in directing NSC fate. Hence, our work aims to dissect how mitochondria biogenesis, morphology and bioenergetics can modulate NSC differentiation. For this, NSCs were obtained by isolating subventricular zone (SVZ) and dentate gyrus (DG) cells from P1-3 C57Bl6 mice[3]. The isolated cells were grown in neurospheres, and consequently passaged to guarantee higher yields of NSCs. Thereafter, neurospheres were plated under specific differentiation conditions giving raise to neurons, astrocytes and oligodendrocytes. Additionally, expression of proteins involved in mitochondrial biogenesis and fusion/fission was determined. Overall, expression of mitochondrial biogenesis-related proteins did not significantly change with NSC differentiation, in both neurogenic niches. Importantly, the levels of proteins involved in mitochondrial fusion (Mfn1/Mfn2) significantly increased while proteins involved in fission (DRP1) significantly decreased along differentiation, only in SVZ cells. Furthermore, mitochondrial number, length and area was different in the different cell types (NSCs and differentiated cells). Indeed, mitochondrial number significantly increased during astroglial and neuronal differentiation. Moreover, both NSCs and oligodendrocyte precursor cells were the cells with more elongated mitochondria. Interestingly, mitochondrial area did not change in

neuronal cells, while there were significant alterations along oligodendroglial differentiation. These results will pave the road towards novel findings concerning the role of mitochondrial dynamics in NSC fate.

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\ POSTER 20 – A novel ketogenic diet slows down seizure development in the rapid kindling rat model of epileptogenesis

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* Shared senior authorship

Abstract:

Background: Patients with drug-resistant epilepsy need alternative therapies in order to adequately treat their recurrent seizures. Although the ketogenic diet (KD) can be an effective treatment option for these patients, the high fat content of the diet introduces tolerability and compliance challenges. To mitigate these issues, we developed a novel KD with a lower fat content and added nutrient combination for neuroprotection, optimized ketogenesis and restriction of glycolysis. The aim is to compare its efficacy to a control diet and classic KD.

Methods: The diets were initiated one week prior to testing seizure development. We used the rapid hippocampal kindling and compared seizure development using the Racine's scale and EEG afterdischarge durations. Blood ketone levels were monitored and behavioral effects of the rapid kindling paradigm and diets were tested in the open field at day 5 after the last kindling.

Results: Both novel and classic KD groups entered ketosis before the onset of rapid kindling, but ketone levels were significantly higher in the classic KD group. The novel KD significantly reduced afterdischarge duration and the rate of seizure progression. The classic KD also shortened after-discharges, but seizure progression was not affected. Rapid kindling increased open field activity and exploration parameters and these were normalized by both KDs.

Conclusions: Inhibition of seizure progression is more effective in the novel KD group compared to the classic KD group, despite the lower ketone levels in the novel KD group than in the classic KD group. This suggests that alternative mode(s) of action could contribute to the observed effects on seizure progression. The novel KD's reduced fat content and enhanced effectiveness in this model of epileptogenesis signify a potentially promising novel therapy for epilepsy patients.

\ PARTICIPANT: CONTACTS, RESEARCH INTERESTS & TECHNICAL EXPERTISE:

Here you can find the contact details, scientific interests and technical expertise of all the participants in the meeting.

Participants will be divided into 4 Working Groups (WG):

- **WG 1 – SYNAPTIC DYSFUNCTIONS & EXCITABILITY IN EPILEPSIES**
- **WG 2 – NEUROINFLAMMATION & GLIAL CELLS IN EPILEPSIES**
- **WG 3 – NOVEL THERAPIES FOR EPILEPSY TREATMENT & DEVELOPMENTAL EPILEPSIES**

Name	e-mail	Scientific Interests	Technics	WG
Alessandro Gaeta (URS)	alessandro.gaeta@uniroma1.it	Epilepsy neurotransmitter receptor GABA ACh	Voltage clamp microtransplation	**1**
Alessandro Mormino (URS)	alessandro.mormino@uniroma1.it	microglia NK cells glioma sleep	RT-PCR, IMMUNOFLUORESCENCE, MACS	**2**
Alessia Romagnolo (AMC-UVA)	a.romagnolo@amsterdamumc.nl	epilepsy, RNA sequencing, microRNA, Focal Cortical Dysplasia (FCD), Tuberous Sclerosis Complex (TSC)	cell culture, RNA sequencing analysis, immunohistochemistry, in situ hybridization	**3**
Ana Sebastião (iMM)	anaseb@medicina.ulisboa.pt	Animal models of epilepsy; excitability control; neuromodulation; Synaptic plasticity; GABA; glutamate	electrophysiology; Neurotransmitter release; neurotransmitter reuptake; uptake; hippocampal slices	**1**
Angelika Muhlebner (AMC-UVA)	a.muhlebner-2@umcutrecht.nl	epilepsy, neuropathology, cortical dysplasia, white matter	histology, cell culture, imaging	**1**
Anwesha Ghosh (iMM)	anwesha.ghosh@medicina.ulisboa.pt	Will update after final approval from my supervisor Prof. Drª Ana Sebastião	Electrophysiology Extracellular, GABA Uptake 3H Radioactive Protocol, Protein Quantification via BioRad DC Protein Assay	**3**

Name	e-mail	Scientific Interests	Technics	WG
Carolina Borlido (iMM)	cborlido@medicina.ulisboa.pt	Alzheimer's disease, CSF and Blood Biomarkers, BDNF, Pharmacology, Organoids	Western Blot, ELISA, Cell Cultures	**2**
Catarina Lourenço (iMM)	catmlourenco@gmail.com	Rett Syndrome, adenosine, BDNF, adenosine augmentation therapy, synaptic plasticity	electrophysiological recordings, molecular techniques, behaviour	**1**
Chiara Dantoni (URS)	chiara.dantoni@uniroma1.it	brain organoids, iPSCs, neurodevelopment, microglia	immunofluorescence, qPCR, confocal microscopy	**1**
Cláudia Valente (iMM)	cvalentecastro@medicina.ulisboa.pt	Neuroinflammation, glia cells, NLRP3 inflammasome, IL-1beta	Western blot, qPCR, Immunohistochemistry, confocal microscopy, primary cultures and slice cultures	**2**
Cristina Limatola (URS)	cristina.limatola@uniroma1.it	microglial cells, neurodegenerative diseases, brain tumors, gut brain interaction, sleep	animal models, cell cultures, microscopy,	**2**
Daniela Abreu (iMM)	daniela.abreu@medicina.ulisboa.pt	Astrocytes, synaptic plasticity, memory, behaviour	Electrophysiology, behaviour	**2**
Daniela Magalhães (iMM)	dmagalhaes@medicina.ulisboa.pt	Neuroinflammation, Glial cells, Behaviour	Behaviour tests, Western blot, ELISA, Immunohistochemestry, Mass spectrometry	**2**
Diogo Lourenço (iMM)	diogo.lourenco@medicina.ulisboa.pt	Neurogenesis; Cannabinoids; Neurodevelopment; Rett Syndrome	Neurosphere cultures; animal behaviour; immunocyto/ histochemistry; PCR	**3**
Eleonora Aronica (AMC-UVA)	e.aronica@amsterdamumc.nl	Epilepsy*epileptogenesis*therapy*development*neuropathology	Neuropathology, transcriptomics, in vitro models	**3**

Name	e-mail	Scientific Interests	Technics	WG
Eleonora De Felice (URS)	eleonora.defelice @uniroma1.it	microglia, synaptic plasticity, LTP,	extracellular field potential recordings, voltage clamp, oocyte microinjection, acute brain slice preparation, RT-PCR,	**2**
Eleonora Palma (URS)	eleonora.palma@ uniroma1.it	epilepsy human neurotrasmitters receptors GABA	electrophysiology voltage-clamp recording patch-clamp	**1**
Frederik Sørensen (AMC-UVA)	frederik1104@hot mail.com	Bioinformatics, Neuroscience, Focal cortical dysplasia, RNAsequencing, Development	scRNAseq, bioinformatics analysis, RNAscope, in situ hybridization	**3**
Gabriel Miltenberger-Miltenyi (iMM)	ggmilten@gmail.c om	Human Genetics, Neurology, Epilepsy, Biomarkers	Sequencing, Variant evaluation, Western Blot, Lipidomics.	**3**
Gabriele Ruffolo (URS)	gabriele.ruffolo@u niroma1.it	Temporal lobe epilepsy; GABA; neurotransmission; neurodevelopment; electrophysiology	two-electrode voltage clamp; membrane extraction; cellular electrophysiology	**3**
Hester Meeusen (AMC-UVA)	Hester.MEEUSEN @external.danone .com	epilepsy, ketogenic diet	cell culture, calcium imaging, Seahorse metabolic assays, immunohistochemistr y	**3**
Isa Fernandes Mota (iMM)	isafernandesmota 99@gmail.com	Neural Stem Cells, Mitochondrial Bioenergetics, Differentiation, Subventricular Zone	Neurosphere culture, Respiratory assays, ATP determination assay, Magnetic Separation, Immunocytochemistry	**3**
James Mills (AMC-UVA)	j.d.mills@amsterd amumc.nl	Bioinformatics epilepsy genomics transcriptomics	RNA-sequencing DNA-sequencing data analysis	**2**
Joana Mateus (iMM)	joana.mateus@me dicina.ulisboa.pt	Neural Stem Cells, Oligodendrocytes, Cannabinoids, BDNF	Animal model of MS, behaviour tests (learning and memory, motor function), SVZ-derived neurosphere cultures, IHC/ICC	**1**

Name	e-mail	Scientific Interests	Technics	WG
João Moreira (iMM)	joaomoreira@medicina.ulisboa.pt	Caffeine; Neurogenesis; Stem Cells, Synaptogenesis; Neurotrophic Factors	Neurosphere primary cultures, immunocyto/histochemistry, fluorescence/confocal microscopy and animal behavior	**1**
Katiuscia Martinello (URS)	katiuscia.martinello@neuromed.it	Temporal lobe epilepsy, inhibitory neurotransmission, Patch-clamp, human brain tissue, excitability	electrophysiology, patch clamp, mouse model of epilepsy	**1**
Laura Ferrucci (URS)	laura.ferrucci@uniroma.it	Neurophysiology, synaptic function, microglia activation	Electrophysiology, Immunofluorescence	**2**
Leonor Rodrigues (iMM)	leonor_rr@hotmail.com	Models of epilepsy, behaviour, EEG	Kainate-induced SE model, Rat behaviour, CSF and blood extraction, perfusions, extracellular recoordings in hippocampal organotypic slices	**3**
Mafalda Manso (iMM)	mafalda.m07@gmail.com	Neuroinflammation, synaptic dysfunction, glial cells	immunohistochemistry, Western-blot, RT-PCR	**2**
Marco Ledri (ULund)	Marco.Ledri@med.lu.se	CRISPR, gene therapy, electrophysiology, epileptogenesis	Optogenetics, electrophysiology, qPCR, Plasmid cloning, vector production	**3**
Maria José Diógenes (iMM)	diogenes@medicina.ulisboa.pt	Rett Syndrome; Alzheimer's Disease; Novel drugs	Neuron primary cultures; Electrophysiology;	**3**
Mariana Neuparth Sottomayor (iMM)	marianasottomayor.mm@gmail.com	Childhood Absence Epilepsy, Astrocytes, GAT-1, Learning and Memory	Behaviour Experiments, fEPSP recordings, Immunohistochemistry, Western Blot	**1**

Name	e-mail	Scientific Interests	Technics	WG
Mariana Van Zeller (iMM)	mariana.campos@medicina.ulisboa.pt	Astrocytes, Alzheimer's disease, neuroinflammation, NLRP3, glial cells	Cell culture, ICC, Western Blot, ELISA	**2**
Marta Morotti (URS)	marta.morotti@uniroma1.it	Microglia, Neuroinflammation, Diseases, Brain	Immunofluorescence, pcr, MACS, RNA extration	**2**
Merab Kokaia (ULund)	merab.kokaia@med.lu.se	Epilepsy, Gene therapy, cell therapy, optogenetics	Electrophysiology, optogenetics, chemogenetics, gene trensfer	**3**
Mirte Scheper (AMC-UVA)	m.scheper@amsterdamumc.nl	TSC, epilepsy, bioinformatics, interneurons	snRNA sequencing analysis, qPCR, in situ hybridization, RNA isolation, cell culture	**3**
Nuno Alemãn (iMM)	nuno.aleman@campus.ul.pt	Biomarker, CSF, Blood, TrkB-ICD, Alzheimer's disease	Western Blot, Murine CSF extraction	**3**
Patrizia Ratano (URS)	patrizia.ratano@uniroma1.it	Endocannabinoid System, Cannabinoids, microglia, inflammation, epilepsy	Rodent behavior, cell culture, rodent neurosurgery, immunofluorescence	**2**
Pierangelo Cifelli (URS)	pierangelo.cifelli@univaq.it	TLE, GABA, GABAa Receptor, epileptogenesis	Electrophysiology	**2**
Ricardo Viais (iMM)	ricardo.viais@gmail.com	Rett Syndrome; Neurodevelopmental epilepsies	Neuron primary cultures; immunofluorescence	**3**
Rita Soares (iMM)	soares.rita123@gmail.com	Mitochondrial Dynamics; Neural Stem Cells; Differentiation; Neurological Disorders	Neurosphere culture; Cell passage; Morphometric analysis; Western Blot; Immunocytochemistry	**3**
Rozemarijn Kalf (AMC-UVA)	rozemarijn.kalf@danone.com	Electrophysiology, dietary treatment, neuroinflammation	IHC, Calcium oscillation assay (primary rat culture), Seahorse assay	**1**

Name	e-mail	Scientific Interests	Technics	WG
Sandra Vaz (iMM)	svaz@medicina.ulisboa.pt	Astrocyte; Absence seizures; neurotransmitters transporters; endocanabinoid system; BDNF;	Calcium signalling; Patch clamp; fEPSPs; behaviour; Stereotaxic Surgery	**1**
Sara Inteiro de Oliveira (iMM)	sara86oliveira@gmail.com	Biology, Neurobiology, Neuroscience, Molecular Biology, Neurodegeneration	Neuron cultures, western blot, electrophysiology, immunocytochemistry	**2**
Sara Paulo (iMM)	sara.paulo@medicina.ulisboa.pt	Adult neurogenesis, plasticity, behaviour, aging, neurodeegneration	Immunohistochemistry, rodent behaviour, intracerebral and intraperitoneal injections, western-blot	**1**
Sara Pinto (iMM)	sfccpinto@gmail.com	excitotoxicity; neuroinflammation; neurodegeneration; glial cells	Electrophysiology; western blot; genotyping	**1**
Sara Xapelli (iMM)	sxapelli@medicina.ulisboa.pt	Neural stem cells, cannabinoids, BDNF, Oligodendrogenesis, Neurogenesis	Neurosphere cultures, Immunhistochemistry, Behaviour, ICV admnistration	**1**
Sergio Fucile (URS)	sergio.fucile@uniroma1.it	ion channels, excitotoxic damage, intracellular calcium, GabaA receptor, nicotinic acetylcholine receptor	Patch-clamp in cells, patch-clamp in brain slices, digital time-resolved fluorescence microscopy	**1**
Tatiana Morais (iMM)	tatianapintomorais@gmail.com	Absence Seizures, GABA, uptake, behaviour, EEG	GABA uptake, surgical cannula implantation, EEG recordings, IHC, microinjection in freely moving animals	**1**

Name	e-mail	Scientific Interests	Technics	WG
Tiago Coelho (iMM)	lapintiago@gmail.com	Alzheimer's disease; BDNF/TrkB-FL signaling; Extracellular vesicles; Proteostasis; Neuron-glia communication	Secretome and extracellular vesicles isolation, western blot, lentiviral transduction, cell culture	**2**
Tiziano D'Andrea (URS)	t.dandrea@uniroma1.it	Neurophysiology, Degenerative Diseases, Excitotoxicity, Epilepsy,	Electrophysiology, Cell Colture, Calcium Imaging	**2**
Vera Neves (iMM)	veraneves@medicina.ulisboa.pt	Blood-brain barrier, drug delivery, peptide, neurosciences, nanomedicine	Blood-brain barrier transport, Blood brain barrier integrity and cytotoxicity, surface-plasmon resonance, atomic force microscopy	**3**

\ POTENTIAL COLLABORATIONS:

One of the aims of EpiEpiNet is to promote and strengthen scientific collaborations among researchers of the Consortium. We give you some pages to take notes on potential collaborations / joint projects /common interests / short-term missions that might emerge.

If you wish so, you can pass on this information to us. We will try to help you in making these ideas come true…

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 952455

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