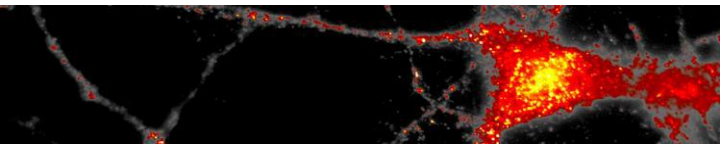
8th ECS Workshop

Calcium Signaling in Aging and Neurodegenerative Diseases

18 - 20 September 2019 | Coimbra, Portugal



ABSTRACT BOOK

european calcium society

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Maria Ankarcrona, Karolinska Institute, Sweden

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Michael Duchen, UCL, UK

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Organization Support

During the conference the Technical Organization will be located at the registration desk.

Email: ecs2019.coimbra@gmail.com
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Free Wi-Fi access will be available at the venue (pass: **trypcoimbra**).

Accreditation

Every participant must pick-up his/her accreditation at the registration desk that is located at the Tryp Hotel's hall. The accreditation must be visible and you should keep it with you during the workshop.

Invited Speakers (Plenary and Symposia)

Please give your presentation to the organization during the registration.

If you need to do any change on your presentation, please contact a member of the Organization.

Certificates

Certificates will be sent by e-mail once the workshop has finished.

ORAL COMMUNICATIONS

Oral presentations will last 10 minutes followed by 5 minutes discussion time.

Please note that you will have to send your presentation on Power Point format before September 18th to ecs2019.coimbra@gmail.com.

POSTERS COMMUNICATIONS

Posters should be prepared in portrait format (width 100 cm, height 160 cm – maximum).

Posters will have to be set-up the first day of the meeting before the opening session (i.e. directly after registration) and will remain on display until the end of the poster session on Friday afternoon. Fixation material for the posters will be available.

Importantly, all posters have to be removed on the Friday, before the start of Session 5.

AWARDS

The winners of "IJMS Best Oral Communication" and "IJMS Best Poster Communication" will be announced during the Final Remarks (Friday 20th, 13h).

IJMS Best Oral Communication	400 €
IJMS Best Poster Communication	300 €

Scientific Programme

WEDNESDAY, SEPTEMBER 18TH

13:00 Reception

14:00 Introduction and welcome

14:30 SYMPOSIUM 1: SYNAPTIC CALCIUM

Chairs: Giles E. Hardingham & Rodrigo Cunha

14:30 Calcium signalling deficits in early Alzheimer's disease pathophysiology

Grace Stutzmann, Rosalind Franklin University. USA

15:10 Modulation of synaptic activity and astrocytic calcium signaling by cannabinoids

Ana Sebastião, IMM. Portugal

15:50 Altered calcium handling and exocytosis in chromaffin cells from mouse models of neurodegenerative diseases

Antonio Garcia. UAM. Spain

16:30 Coffee break and poster session

18:00 PLENARY LECTURE:

Ca^{2+} signaling pathways to degeneration or resilience in neurons and astrocytes

Giles E. Hardingham, The University of Edinburgh. UK

19:00 Presentation of Junior ECS Board

19:15 Welcome cocktail at the Venue

THURSDAY, SEPTEMBER 19TH

8:30 SYMPOSIUM 2: MITOCHONDRIAL CALCIUM

Chairs: Ana Cristina Rego & Catarina Oliveira

8:30 Linking mitochondrial dysfunction to neurodegeneration: impaired mitochondrial bioenergetic capacity sensitises neurons to calcium overload

Michael Duchen, University College London. UK

9:10 TOM70: a new player regulating Ca2+-transfer at the endoplasmic reticulum-mitochondria signaling platform

Maria Ankarcrona, Karoliska Institute. Sweden

9:50 Mechanisms underlying regulation of mitochondrial quality by the PINK1/Parkin pathway and consequence of their dysfunction

Olga Corti, Brain and Spine Institute. France

10:30 Coffee break

11:00 ORAL COMMUNICATION – SESSION 1

11:00 In vivo Ca^{2+} imaging reveals correlation between sarcoplasmic reticulum Ca^{2+} content and sarcopenia in aging flies

Delrio-Lorenzo A, Rojo-Ruiz J, Alonso T and García-Sancho J

11:15 Mitochondrial Ca^{2+} signaling and bioenergetics dysregulation in App knock-in mice: relevance of ER-mitochondria contacts and therapeutic opportunities

Naia L, Pinho CM, Leal NS, Liu J, Li X, Dentoni G, Shimozawa M, Connolly NMC, Pizzo P, Prehn J, Nilsson P, Ankarcrona M

11:30 Inhibition of Sarco-Endoplasmic Reticulum Ca2+ ATPase extends the lifespan in C. elegans worms

García-Casas P, Arias del Val J, Alvarez-Illera P, Fonteriz RI, Montero M, Alvare J

11:45 Extra-synaptic NMDA receptors, b-amyloids and neuronal activity

Dupont G, Gall D

12:00 Activation of TRPC1 channel by mGluR5 modulates synaptic plasticity, spatial working memory and memory extinction

Gualdani R, Lepannetier S, Yerna X, Schakman O, Gailly P

12:30 Lunch and poster session

13:30 SYMPOSIUM 3: ER CALCIUM

Chairs: Cláudia Pereira & Jan B. Parys

13:30 Calcium signaling in zebrafish and mice models of neurodegeneration

Jacek Kuznicki, IIMCB. Poland

14:10 Calcium remodeling in aging and Alzheimer´s disease

Carlos Villalobos, SCIC. Spain

14:50 Presenilin 2 and ER Ca2+ handling: functional consequences for neurodegeneration

Paola Pizzo, University of Padova. Italy

15:30 Coffee break and poster session

16:00 ORAL COMMUNICATION – SESSION 2

16:00 Store-operated calcium channels control neural stem cell self-renewal in the adult brain subventricular zone

Constantin B, Domenichini F, Arnault P, Terrié E, Harnois T, Magaud C, Bois p, Coronas V

16:15 Role of the purinergic P2X7 receptor on intracellular calcium signaling in peripheral blood mononuclear cells of patients with Major depressive disorder

Garrosa J, Lopez García P, López MG, García AG, Cano-Abad MF

16:30 Effect of Hereditary Spastic Paraplegia genes on presynaptic calcium handling

Oliva MK, Perez-Moreno J, O'Shaughnessy J, Wardill TJ, O'Kane CJ

16:45 Mesmerize: A powerful highly versatile, expandable, and self-documenting platform for calcium imaging analysis

Kolar K, Chatzigeorgiou M

17:00 Shedding light on intracellular organelle contacts with novel modular genetically encoded sensors

Vallesea F, Catoni C, Ramirez O, Barazzuol L, Bading H, Brini M, Calì T

18:00 Social Event

20:00 Conference Dinner

FRIDAY, SEPTEMBER 20TH

9:00 SYMPOSIUM 4: CALCIUM IN GLIA AND STEM CELLS

Chairs: João Malva

9:00 Calcium-dependent mechanisms of cerebral blood flow regulation

Martin Lauritzen, University of Copenhagen. Denmark

9:40 Calcium-dependent signaling in glioblastoma stem-like cells

Catherine Leclerc, Center for Integrative Biology. France

10:30 Coffee break and poster session

11:30 SYMPOSIUM 5: TECHNICAL APPROACHES ON CALCIUM IMAGING

Chairs: Luisa Cortes & Carlos Villalobos

11:30 Shining light on hierarchical cortical interactions

Leopoldo Petreanu, Champalimaud Foundation. Portugal

12:10 GAP, flies, sarcoplasmic reticulum and aging

Javier García-Sancho, University of Valladolid. Spain

12:50 Final Remarks

13:15 ECS General Assembly

INVITED ORAL COMMUNICATION ABSTRACTS

SYMPOSIUM 1 - SYNAPTIC CALCIUM

IOC1: Calcium signalling deficits in early Alzheimer's disease pathophysiology

Mustaly S.[a,b], Schrank S.[a,b], Chakroborty S.[b], McDaid J.[a,b], Marr R.[a,b], Stutzmann G.[a,b]

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[b] Department of Neuroscience, Chicago Medical School, RFUMS, North Chicago, IL USA

Despite the increasing prevalence of Alzheimer's disease and related dementias, there are no effective treatments that protect cognitive function and the underlying causes remain elusive. This in part may reflect an incomplete understanding of proximal disease mechanisms, particularly those that directly affect learning and memory encoding. Furthermore, discrepancies between animal models used to validate therapeutics and disease processes in human neurons have hindered progress.

Here, we examine several neuronal signaling and protein handling cascades in AD mouse models and transformed neurons from AD patients to identify shared and divergent pathogenic cascades, and validate novel therapeutic targets in human AD neurons. Using indirectly (via iPSCs) and directly transformed neurons from human AD patients and age-matched controls, and acute brain slices from 3xTg-AD and PS1/APP mice, we examine upstream intracellular Ca^{2+} pathways at various disease stages to examine their role in synaptic structure and function, synaptic plasticity encoding, and in organelles important for protein handling and cellular metabolism. This is accomplished using live cell imaging and electrophysiological recordings of neurophysiological properties and synaptic function, and immunoassays of pathogenic protein species in the mouse and human neural networks.

We identified several key upstream pathogenic mechanisms in human AD neurons that are also present in AD mouse models. These include significantly increased ER-Ca^{2+} release through ryanodine receptors (RyR), increased expression of the RyR2 isoform, increased phospho-tau species, altered synaptic plasticity expression, and defects in lysosomal pH and clearance of aberrant proteins. Incubation in RyR-modulating compounds prevented excess Ca2+ release in the human AD neurons, and reversed related downstream pathology such as synaptic defects, amyloid and tau aggregation, lysosomal and mitochondrial dysfunction, and other Ca2+-regulated events that drive AD-associated memory decline.

Thus, aberrant calcium signaling presents as an early and upstream driver of many of the central features of AD, particularly those regulating memory encoding, and may be a novel target for disease-modifying therapeutic strategies. Furthermore, utilizing clinically-relevant models to investigate AD, such as human neurons derived from AD patients, can reveal critical and clinically relevant pathogenic mechanisms by which to validate novel targets.

IOC2: Modulation of synaptic activity and astrocytic calcium signalling by cannabinoids

Sebastião AM[a,b,ɟ], *Silva-Cruz A*[a,b], *Ribeiro JG*[a,b], *Vaz SH*[a,b], *Mouro FM*[a,b], *Ribeiro JA*[a,b]

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Cannabinoid receptors 1 (CB1R) are widely distributed in excitatory and inhibitory neurons, and in astrocytes. Exogenous activation of CB1R inhibit excitatory and inhibitory synaptic transmission, to inhibit long-term potentiation (LTP) of synaptic transmission and to disrupt memory. Adenosine is another ubiquitous neuromodulator of synaptic signalling. We have been focusing on 1) understanding the role of endocannabinoids (eCBs) to modulate synaptic plasticity phenomena; 2) understanding how the two neuromodulators, cannabinoids and adenosine, control each other; 3) identifying the actions of these neuromodulators on the 3rd component of the tripartite synapses – the astrocytes. We found out that eCBs have a dual role upon hippocampal LTP, inhibiting weak LTP while facilitating strong LTP (Silva-Cruz et al., 2017 - Front Pharmacol. 8:921.eCollection), likely acting as a high pass filter to reduce signal to noise ratio of synaptic strengthening during memory consolidation. Exogenous activation of CB1Rs consistently inhibit LTP at the hippocampus and, probably by disrupting the fine-tune homeostatic control exerted by eCBs upon synaptic plasticity, consistently disrupt memory consolidation and affect brain connectivity between brain areas relevant for memory (Mouro et al., 2018 - J Neurochem 47:71-83). Importantly, adenosine A_{2A} receptor ($A_{2A}R$) antagonists are able to attenuate the inhibitory action of CB1R agonists upon LTP and prevent memory consolidation impairment caused by either acute (Mouro et al., 2017 - Neuropharmacology, 117:316-327) or chronic (Mouro et al., 2019 - Neuropharmacology 155:10-21) intake of CB1R agonists. A1Rs, though present in CB1R positive interneurons (Rombo et al., 2016 - Cereb Cortex 26:1081-1095), do not influence the inhibitory action of exogenous activation of CB1Rs on synaptic transmission (Serpa et al., Eur J

Pharmacol. 623:41-46) or plasticity (Silva-Cruz et al., 2017 - Front Pharmacol. 8:921.eCollection). In astrocytes CB1R activation leads to intracellular calcium oscillations and to facilitation of glutamate uptake, both actions being also under control of adenosine A2ARs (work in progress). In conclusion, cannabinoids and adenosine affect calcium-dependent activity of the tripartite synapse, and interactions between these two neuromodulators impact in cognitive phenomena. The interaction between CB1Rs and A2ARs may prove relevant when mitigation of cognitive deficits during cannabinoid-based therapies is required.

Keywords: Endocannabinoid signalling; Cannabinoid receptors; Adenosine receptors; Synaptic plasticity; astrocyte calcium signalling

Funding: Work supported by EU H2020 (SynaNet, project number: 692340), FCT (PTDC/DTP-FTO/3346/2014) and Cardlane Ltd. The supply of A1R-KO mice by M Carlstrom (Karolinska Inst) and of KW-6002 by C Muller (Bonn Univ.), is also acknowledged.

IOC3: Altered calcium handling and exocytosis in chromaffin cells from mouse models of neurodegenerative diseases

De Diego AMG[a,b] and Garcia AG[a,b]

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Synaptic disruption and altered neurotransmitter release occurs in the brain of patients and murine models of neurodegenerative diseases (NDDs). During the last years, evidence has accumulated suggesting that the sympatho-adrenal axis is also affected as disease progresses[1,2]. For instance, α-synuclein that is associated with Parkinson's disease (PD) decreases exocytosis and promotes fusion pore dilation in adrenal chromaffin cells (CCs)[3]. Furthermore, Huntington-associated protein 1 (HAPI) interacts with huntingtin that, when mutated, causes Huntington's disease (HD); HAPI reduces full fusion exocytosis by affecting vesicle docking and controlling fusion pore stabilization[4]. HAPI also decreases endocytosis by binding to clathrin[5]. In R6/1 mouse models of HD, mutated huntingtin is overexpressed in CCs; this causes decreased quanta! secretion, smaller quantal size and faster kinetics of the exocytotic fusion pore, pore expansion, and closure; this was accompanied by decreased sodium current, decreased acetylcholine-evoked action potentials, and attenuated $[Ca^{2+}]c$ transients with faster Ca^{2+} clearance[6]. In the SODI G93A mouse model of amyotrophic lateral sclerosis (ALS), CCs exhibited secretory single-vesicle spikes with slower release rate but higher exocytosis[7]. Finally, in the APP/PSl mouse model of Alzheimer's disease (AD), the stabilization, expansion, and closure of the fusion pore was faster but the secretion was attenuated[8]. Alterations here described are consistent with the hypothesis that central alterations undergone in various NDDs are also manifested at the peripheral sympatho-adrenal axis to impair the stress fight-or-flight response in patients suffering those diseases. Such alterations may occur: (i) primarily by expression of mutated disease proteins in CCs; (ii) secondarily to stress adaptation imposed by disease progression and the limitations of patient autonomy.

Keywords: Chromaffin cell, neurodegenerative disease, sympatho-adrenal, exo-endocytosis

Funding: *Spanish Ministry of Science, Technology and Innovation SAF2016-78892R; EU Horizon 2020 Research and Innovation Programme under Marie Slodowska-Curie Grant Agreement N. 766124.*

References:

1. de Diego & Garcia, *Acta.Physio*. (2018).
2. Carbone, et al. *Comprehensive Physiology* (2019)
3. Logan, et al. *Nat. Neurosci.* (2017).
4. Mackenzie et al. *J.Physiol*. (2014).
5. Mackenzie et al. *Cell. Signal.* (2017).
6.Martfnez-Ramfrez, et al. *J. Neurochem*. (2018).
7.Calvo-Gallardo et al. *Am.J.Physiol., Cell Physiol.* (2015).
8. de Diego et al. *Biochem.Biophys. Res.Commun*. (2012).

PLENARY CONFERENCE

IOC4: Ca^{2+} signaling pathways to degeneration or resilience in neurons and astrocytes

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Our laboratory is focussed in the molecular mechanisms of core interdependent pathological processes upon which several neurological disorders converge, including oxidative stress, mitochondrial dysfunction, inflammation, and aberrant NMDA receptor activity. On the flip side, we are interested in the brain's capacity for homeostasis, particularly the ability of neurons and astrocytes to mount adaptive protective responses to adverse conditions, through both inter- and intra-cellular signaling. As will be discussed, the second messenger Ca^{2+} plays a pivotal role in both degenerative and protective pathways in the CNS.

SYMPOSIUM 2 – MITOCHONDRIAL CALCIUM

IOC5: Linking mitochondrial dysfunction to neurodegeneration: Impaired mitochondrial bioenergetic capacity sensitises neurons to calcium overload

Nicoletta Plotegher and Michael R Duchen
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Parkinson's Disease (PD) destroys lives, but there are no disease modifying treatments and we are reliant on symptomatic control.

Extensive data point to a major role for impaired mitochondrial function in the pathophysiology of PD. Genetic forms of the disease implicate defects in pathways of autophagy or mitophagy, processes which play an essential role in the maintenance of mitochondrial health and energy homeostasis. Heterozygous mutations of the lysosomal enzyme glucocerebrosidase (GBA) have emerged as the major genetic risk factor for PD, while homozygous GBA mutations cause Gaucher disease (GD), a lysosomal storage disorder which includes a severe neuronopathic form. We have explored the physiology of primary neurons cultured from the CNS of GBA knockout mice, and found that loss of GBA function is associated with severe mitochondrial dysfunction, including a decreased mitochondrial membrane potential, decreased respiratory rate and decreased maximal respiratory capacity. Exploring the mechanisms that link impaired lysosomal function and mitochondrial dysfunction, we found that both autophagy and the proteosomal degradation pathway were profoundly downregulated[1].

We have now explored the impact of mitochondrial dysfunction on cell physiology in the same model system. We explored the cellular response to a glutamate induced calcium signal imposing a demand for increased work. In control cells, physiological glutamate concentrations caused a transient Calcium signal, and a transient decrease in ATP/ADP ratio, measured with the probe 'perceval'[2] that recovered within 10 s of seconds. In contrast, in the

gba$^{-/-}$ cells, ATP/ADP fell irreversibly in response to even 1uM glutamate, associated with deregulation of calcium homeostasis and mitochondrial depolarization. Thus, the lack of bioenergetic capacity to respond to increased energy demand with increased ATP supply renders the neurons vulnerable to otherwise innocuous concentrations of glutamate, which may play a role in neurodegeneration. Strikingly, *gba1*$^{+/-}$neurons showed a behavior that was similar to that of *gba1*$^{-/-}$, consistent with a role of these mechanisms in the pathogenesis of PD. These data reveal the fundamental importance of mitochondrial bioenergetic capacity in protecting cells from dysregulation of cellular calcium homeostasis.

1. Plotegher, N & Duchen, MR (2017) *Trends in Molecular Medicine*;23(2):116-134
2. Berg J, Hung YP, Yellen G. (2009) *Nat Methods*; 6(2):161-6.

Keywords: glutamate; mitochondria; glucocerebrosidase

Funding: *supported by Marie Curie Actions and Michael J Fox Foundation*

IOC6: TOM70: a new player regulating Ca^{2+}-transfer at the endoplasmic reticulum-mitochondria signaling platform

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Intracellular cross-talk and signaling take place at specialized contact sites between organelles including endoplasmic reticulum (ER) and mitochondria, ER and plasma membrane, ER and endosomes. Interestingly, many cellular defects implicated in different diseases, including cancer and neurodegenerative diseases, can be linked to dysfunctions in ER and mitochondria. ER and mitochondria interact with each other at specialized domains on the ER called mitochondria-associated membranes (MAMs) which serve as exchange platforms for calcium (Ca^{2+}), metabolites and lipids, all critical for the function of both organelles.

The mitochondrial translocase of the outer membrane (TOM) is a protein complex essential for the post-translational import of nuclear-encoded mitochondrial proteins. Among its subunits, TOM70 and TOM20 are only transiently associated with the core complex, suggesting their possible additional roles within the outer mitochondrial membrane (OMM). Here, by using different mammalian cell lines, we demonstrate that TOM70, but not TOM20, clusters in distinct OMM foci frequently overlapping with sites in which ER contacts mitochondria. Functionally, TOM70 depletion specifically impairs IP3-linked ER to mitochondria Ca^{2+} transfer. This phenomenon is dependent on the capacity of TOM70 to interact with IP3-receptors and favour their functional recruitment close to mitochondria. Importantly, the reduced constitutive Ca^{2+} transfer to mitochondria, observed in TOM70 depleted cells, dampens mitochondrial respiration, affects cell bioenergetics, induces autophagy and inhibits proliferation. Our data reveal a hitherto

unexpected role for TOM70 in pro-survival ER-mitochondria communication, reinforcing the view that the ER-mitochondria signaling platform is a key regulator of cell fate.

This novel role of TOM70 has so far not been investigated in disease. Since our laboratories are interested in cellular mechanisms underlying neurodegeneration and in particular Alzheimer's disease (AD) we have recently initiated such studies. Preliminary data from human postmortem samples and several AD mouse models reveal that TOM70 protein levels are specifically decreased. A lower TOM70 expression may lead to impairment of mitochondrial protein import as well as effects on Ca^{2+} transfer from ER to mitochondria as shown previously in cell lines by our laboratories. Studies to elucidate the role of TOM70 in AD initiation and progression are underway.

Keywords: TOM70, endoplasmic reticulum, mitochondria, calcium, neurodegeneration

IOC7: Mechanisms underlying regulation of mitochondrial quality by the PINK1/Parkin pathway and consequence of their dysfunction

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Mitochondrial quality control is orchestrated by a series of fundamental mechanisms aimed at preserving mitochondrial activity and adapting it to the changing environment in order to promote cell survival. The relevance of these mechanisms is highlighted by the dramatic consequence of the genetic alteration of intrinsic or regulatory components of these pathways. One example is illustrated by loss-of-function mutations in the *PARK2* and *PINK1* genes, which lead to autosomal recessive forms of Parkinson's disease. These genes encode the E3 ubiquitin ligase Parkin and the mitochondrial serine/threonine kinase PINK1, which jointly regulate various mitochondrial quality control mechanisms, in particular the degradation of dysfunctional mitochondria by autophagy, a process termed mitophagy. Despite the remarkable advances in our understanding of the molecular mechanisms by which these proteins cooperate in sensing mitochondrial damage and activating mitophagy, whether dysfunction of this pathway is central to neuronal degeneration in Parkinson's disease remains debated. I will present recent efforts from our laboratory to investigate more broadly the consequence of dysfunction of the PINK1/Parkin system in different cell types from Parkin-deficient mice and patients with *PARK2*-linked Parkinson's disease. Our studies in primary fibroblasts, neurons and glial cells highlighted defects in the endoplasmic reticulum (ER)-mitochondrial interface, ER-to-mitochondria calcium transfer, mitophagy, the response to mitochondrial stress, mitochondrial import and the NLRP3 inflammasome pathway in cells with dysfunctional Parkin. I will discuss the possibility that PINK1/Parkin-dependent mechanisms play a role beyond autosomal recessive Parkinson's disease.

Keywords: PINK1/Parkin pathway, *mitophagy*, *Parkinson's disease*

Funding: We received support from Institut national de la santé et de la recherche médicale (INSERM), Fondation Institut du Cerveau et de la Moelle épinière and Agence Nationale pour la Recherche ("Investissements d'avenir" grant ANR-10-IAIHU-06), AETIONOMY (Innovative Medicines Initiative Joint Undertaking under grant agreement N°115568, European Federation of Pharmaceutical Industries and Associations companies' in kind contribution), Fondation de France, Association France Parkinson, Michael J. Fox Foundation.

SYMPOSIUM 3 - ER CALCIUM

IOC8: Calcium signaling in zebrafish and mouse models of neurodegeneration

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Ageing of human population generates new challenges regarding age-related diseases such as Alzheimer's (AD), Parkinson's disease (PD) or cancer. To find new drug targets and treatments badly needed for neurodegenerative disorders more basic research is required. One of the approaches in this type of research is to generate animal models of a particular disease and to characterize their pathological features. Our research is focused on AD, PD and Huntington's (HD) disease. We study calcium homeostasis and signalling in the zebrafish and mouse models of these diseases. In particular, we analyse zebrafish line with *pink1* mutant as a model of PD. The observed loss of dopaminergic neurons in this fish can be rescued by removal of mitochondrial calcium uniporter (Mcu). The *mcu* knockout line is fertile, has no calcium influx into mitochondria, but no loss of dopaminergic neurons. We found that *mcu* knockout fish is insensitive to MPTP, a known toxin of these neurons. MPTP treatment of wild type fish leads to the loss of dopaminergic neurons and is often used to generate another PD model. To check calcium hypothesis of AD we generated mice with overexpression of either STIM1, STIM2 or Orai1 and checked behaviour of aged animals. A battery of behavioural tests showed that context recognition decreased only in female Orai1 transgenic mice, but not in males. The epileptic-like phenotype that was observed in female mice suggests that ORAI1 overexpression may affect neuronal activity in a sex-dependent manner. On the other hand, STIM1 overexpression in neurons in the brain perturbed metabotropic glutamate receptor signaling, leading to impairments in long-term depression and alterations in animal behaviour. In mice overexpressing huntingtin mutant with 126 glutamine

residues (YAC128) we found that dysregulation of calcium homeostasis correlates with changes in the gene expression of members of the calciosome, especially increased expression of Hap1. Our results showed that HAP1A causes the activation of Store Operated Calcium channels in HD models by affecting IP_3R1 activity. In summary, the models of neurodegenerative diseases help to understand the molecular mechanisms involved in changes of calcium homeostasis which occur in human pathologies.

Keywords: Alzheimer's, Parkinson's, Huntington's diseases, calcium homeostasis

Funding: OPUS grant (2016/23/B/NZ3/03142) and MAESTRO Grant (2011/02/A/NZ3/00144) to JK from the National Science Centre

IOC9: Calcium remodelling in aging and Alzheimer´s disease

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Intracellular Ca^{2+} homeostasis plays an important role in control of neuronal activity including neurotransmitter release, synaptic plasticity and memory storage as well as neuron cell death. Aging is often associated to cognitive decline and it is the most important risk factor for neurodegenerative disorders, particularly Alzheimer's disease (AD). AD is associated to excess of neurotoxic oligomers of amyloid β peptide (Aβo) but the action mechanisms are still highly controversial. We have recently shown that long-term cultures of rat hippocampal neurons, that resemble in many aspects aging neurons, undergo cell death after treatment with Aβo, whereas short-term cultures resembling young neurons do not. In search for the mechanisms involved in this age-related susceptibility to damage, we have shown that in vitro aged neurons undergo a deep remodelling of intracellular Ca^{2+} homeostasis. Specifically, aging neurons show increased resting cytosolic Ca^{2+} concentration, enhanced Ca^{2+}store content and Ca^{2+} release from intracellular stores as well as increased Ca^{2+} transfer from the endoplasmic reticulum (ER) to mitochondria. Aged neurons also show decreased store-operated Ca^{2+} entry (SOCE), a Ca^{2+} entry pathway related to memory storage. At the molecular level, this Ca^{2+} remodelling is associated to changes in the expression of Ca^{2+} channels including NMDA receptor isoforms, IP_3 receptor isoforms, the mitochondrial calcium uniporter (MCU) and Orai1/Stim1, the molecular players involved in SOCE. We have also shown recently that Aβo, the most likely neurotoxin involved in AD, exacerbate most of these changes observed in aged neurons leading to enhanced cell death, or to increased

susceptibility to cell death induced by insults like NMDA and LPS. Interestingly, we have shown that Aβo increases Ca^{2+} transfer from ER to mitochondria in young neurons but have no detrimental effects. Aβo also increase colocalization of ER and mitochondria in young and aged neurons. However, in aged neurons, Aβo suppress transfer of Ca^{2+} from ER to mitochondria, decrease mitochondrial potential, enhance generation of reactive oxygen species (ROS) and induce apoptosis. Accordingly, we have proposed that Aβo may be a physiological modulator of ER-mitochondria coupling in hippocampal neurons. However, the excess of Aβo jointly with the age-related remodelling of intracellular Ca^{2+} homeostasis may lead to loss of ER-mitochondrial coupling and contribute to AD.

Keywords: Aging, Alzheimer´s disease, calcium remodelling, amyloid oligomers, mitochondria

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IOC10: Presenilin 2 and ER Ca^{2+} handling: functional consequences for neurodegeneration

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Alzheimer's Disease (AD) is the most frequent cause of dementia in the elderly. Few cases are familial (FAD), due to autosomal dominant mutations in presenilin-1 (PS1), presenilin-2 (PS2) or amyloid precursor protein (APP). The three proteins are involved in the generation of amyloid-beta (Ab) peptides, providing genetic support to the hypothesis of Ab pathogenicity. However, FAD-PS mutants have been also reported to cause Ca^{2+} dysregulation, independently from their enzymatic activity involved in Ab peptide production, and defective Ca^{2+} handling represents a common feature of several neurodegenerative diseases, including AD. In particular, FAD-PS2 mutants dampen ER Ca^{2+} content and potentiate ER-mitochondria juxtaposition. These effects have a profound influence on different key cell functionalities, such as autophagy, mitochondrial activity and dynamics. Indeed, FAD-PS2 expressing cells show a blocked autophagy, due to an impairment in autophagosome-lysosome fusion. Moreover, *in vivo* mitochondrial axonal transport results also affected by FAD-PS2 expression. Finally, mitochondrial respiration and ATP production are reduced, suggesting an unbalanced cell metabolism, potentially connected to neurodegeneration.

The possibility that the above alterations could be linked to the pathogenicity of FAD-PS2 mutants is discussed.

Keywords: Presenilin, Calcium, ER, Alzheimer's disease, mitochondria

SYMPOSIUM 4 – CALCIUM IN GLIA AND STEM CELLS

IOC11: Calcium-dependent mechanisms of cerebral blood flow regulation)

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Brain's electrical activity correlates strongly to changes in cerebral blood flow (CBF) and the cerebral metabolic rate of oxygen ($CMRO_2$). Brain oxygen consumption is correlated and controlled by Na,K-ATPase activity driven by transmembrane currents, while Ca^{2+}-signals control activity-dependent rises in CBF. In the past, brain capillaries were viewed as tubes that passively conducted blood from the heart to the active nerve cells. However, recent data provided by others and by us suggest that brain capillaries may have control mechanisms for blood flow regulation, and novel microscopy techniques have now made it possible to examine the blood-brain barrier (BBB) in more detail in living animals as well. I here report that rises in synaptic activity in mouse somatosensory cortex evoke capillary dilation that mostly starts in capillaries before arterioles. The capillary dilation is initiated at precapillary sphincters and 1st or 2nd order capillary, from where it propagates upstream to the penetrating arteriole and downstream to higher order capillaries. Application of the gliotransmitter ATP induces dilation followed by constriction that also propagates up- and downstream at velocities of 5-20 µm/s, i.e. at a similar speed as an intracellular calcium wave. In pathology, e.g., cortical spreading depolarization (CSD), which is thought to trigger migraine in patients, the sphincter and capillaries contract, decreasing the lumen and increasing the length of the segment during the period of low blood flow that concurs with migraine symptoms. In normal function and CSD the fluctuations of vascular diameters are contra-correlated to cytosolic Ca^{2+}

in vascular smooth muscle and bran pericytes. In the case of cardiac arrest, precapillary sphincters and capillaries demonstrate a total collapse ~10-20 min after onset of ischemia. This timing is similar to the timing of the so-called 'no-reflow' phenomenon in global ischemia. Our study provides uniques insights into the correlation between Ca^{2+} rises in nerve cells and vascular cells as key to an understanding of brain vascular control.

Keywords: Glutamate, calcium, blood flow, vascular smooth muscle, pericytes

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IOC12: Calcium-dependent signalling in glioblastoma stem-like cells

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Glioblastomas (GBM) are the most aggressive and lethal primary astrocytic tumors in adults, with very poor prognosis. GBMs display significant heterogeneity within the tumor mass, among which a small sub-population of tumor cells with stem-like properties (GSLCs) is responsible for tumor growth, resistance to therapies and tumor recurrence. The high resistance of GSLCs to chemotherapies have been attributed to their capacity to enter quiescence. Quiescence is a reversible cell-cycle arrest which allows cancer stem-like cells to evade killing following therapies. By combining bioluminescent Ca^{2+}-imaging and RNAseq analysis we explored how proliferating and quiescent GSLCs maintain Ca^{2+} homeostasis and showed that the remodelling of the Ca^{2+} homeostasis and the reshaping of mitochondria might favour quiescent GSLCs' survival and aggressiveness in glioblastoma. The importance of the role played by Ca^{2+} in these last processes lead us to search for Ca^{2+}-binding proteins which can relay Ca^{2+} signaling to gene expression. KCNIPs (potassium channel-interacting proteins), which constitute the class E of Ca^{2+} sensor family can control gene transcription directly by binding, *via* a Ca^{2+}-dependent mechanism, to specific DNA sites on target genes. The presence of putative binding sites for KCNIPs on genes associated with unfavorable outcome for GBM patients suggests that KCNIP proteins may contribute to the alteration of the expression of these prognosis genes. This presentation will also discuss the role of this multifunctional Ca^{2+} sensors in the regulation of Ca^{2+}

homeostasis in glioblastoma and how Ca^{2+} via KCNIP proteins may affect prognosis genes expression in GBM.

Keywords: Glioblastoma stem-like cells, KCNIP proteins, mitochondria, Ca^{2+}-aequorin imaging, RNAseq.

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SYMPOSIUM 5 – TECHNICAL APPROACHES ON CALCIUM IMAGING

IOC13: Shining light on hierarchical cortical interactions

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The neocortex is organized in a hierarchy of functionally specialized areas. This architecture influences our understanding of sensory, motor and cognitive processes and inspires machine learning algorithms. Several theories suggest how the multi-level network of the neocortex might allow us to build complex representations of the world, use prior experience to interpret current events, predict the consequence of our actions or learn from experience. However, the computations that the hierarchical organization of the neocortex network endows us with remain largely unknown. This in large part due to the lack of understanding of organizing rules in cortico-cortical connections that could inspire and constrain theories of cortical computation. Using calcium imaging in afferent axons, we measured the organization of cortico-cortical connections in mouse visual cortex. We found that long-range cortical connections are organized with tuning-dependent specificity following simple geometrical rules. Our observations shine light on the computations implemented by long-range cortical networks, constraining existing theories of cortical computations.

Keywords: Two photon imaging, feedback, mouse, visual cortex, axons

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IOC14: GAP, flies, sarcoplasmic reticulum, calcium and aging

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The interest on Ca^{2+} signaling is moving from the cytosol to the organelles, as proper organellar function often requires proper control of luminal Ca^{2+} homeostasis. Subcellular Ca^{2+} signals in organelles are best monitored using genetically encoded calcium indicators (GECI) targeted to the different organelles. Aequorins (AEQ) were the first GECI used as Ca^{2+} probes, and novelties will be treated here, including low affinity variants of AEQ and fluorescent fusion proteins with GFP such as GAP (for GFP-Aequorin-Protein) (1-3). GAP exhibits a unique combination of favourable features: dual-excitation ratiometric imaging capability, high dynamic range, good signal-to-noise ratio, insensitivity to pH and Mg^{2+}, uncomplicated calibration, and targetability to different organelles. Low affinity GAP variants have been engineered for measuring accurately in the high $[Ca^{2+}]$ compartments, such as ER or Golgi (3). On the other hand, GAP was expressed in a variety of organs and tissues including nervous system. As neither AEQ nor GFP are present in mammalian cells, perturbations of cell physiology by interactions with endogenous ligands do not apply, and transgenic animals expressing ER-targeted GAP do not show phenotypic alterations. We shall provide proof of concept for the suitability of the new biosensors to monitor Ca^{2+} dynamics inside intracellular organelles, including ex vivo and in vivo measurements in transgenic animals. Sarcoplasmic reticulum Ca^{2+} dynamics in the muscle of living flies will be studied with special care. A procedure for Ca^{2+} calibration and normalization in the living muscle will be shown. By using this procedure we can study the changes of the SR Ca^{2+} content with aging. Our results show that the decrease of muscle activity with aging correlates very well with a parallel decrease of $[Ca^{2+}]_{SR}$. This Ca^{2+} dishomeostasis derives from a disruption of the pump-leak steady state, with a moderate decrease of SERCA expression, no modification of the ryanodine receptors and increase of SR Ca^{2+} leakiness.

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Keywords: Sarco/*Endoplasmic Reticulum, Calcium, Drosophila, Aging*

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SELECTED ORAL COMMUNICATION ABSTRACTS

SESSION 1

OC1: *In vivo* Ca^{2+} imaging reveals correlation between sarcoplasmic reticulum Ca^{2+} content and sarcopenia in aging flies

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A hallmark of aging is the progressive decline in skeletal muscle mass and function, a process known as sarcopenia. This age-dependent loss of muscle mass and function occurs in humans as well as in invertebrates, including the fly *Drosophila melanogaster*. Skeletal muscle contraction is activated by depolarization of the sarcolemma and Ca^{2+} release from the sarcoplasmic reticulum (SR) to the cytosol. Cytosolic Ca^{2+} binds troponin C resulting in muscle contraction by sliding of the myofilaments. This Ca^{2+}-dependent process, known as excitation-contraction (EC) coupling, has been proposed to be impaired in aging. Whereas the cytosolic Ca^{2+} has been widely studied, little is known about the role of Ca^{2+} dynamics within the SR in muscle excitation. Here we explored the hypothesis that the reduction of the SR Ca^{2+} levels is at the origin of the Ca^{2+} dyshomeostasis in aging. We use the fly *Drosophila melanogaster* as a model to investigate the relationship among lifespan, motor function and SR Ca^{2+} dynamics. We developed a novel quantitative calibrated method to measure SR Ca^{2+} content, based on a low affinity ratiometric Ca^{2+} sensor (GAP3) targeted to the SR of thoracic muscles or the endoplasmic reticulum (ER) of brain neurons. We found that resting muscle $[Ca^{2+}]_{SR}$ content decreases progressively and significantly with age, and this fact correlates with muscle loss of function, measured by the climbing assay. These results can be partially explained by the reduced protein levels of Sarco-Endoplasmic Reticulum Ca^{2+} -ATPase (SERCA) in aged muscles. By contrast, the $[Ca^{2+}]_{ER}$ levels in brain neurons did not change with age, in

agreement with the stable SERCA protein levels in this tissue. Moreover, we found no significant difference in the expression of the Ryanodine receptor (RyR) in both studied tissues. These findings suggest that age-dependent Ca^{2+} dyshomeostasis is tissue specific and, more importantly, our results support the idea of increased SR leakiness in aging, pointing to SERCA as the key player in $[Ca^{2+}]_{SR}$ content.

Keywords: Aging, in vivo, Ca^{2+} imaging, fly, sarcoplasmic reticulum

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OC2: Mitochondrial Ca^{2+} signaling and bioenergetics dysregulation in *App* knock-in mice: relevance of ER-mitochondria contacts and therapeutic opportunities

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The functional interaction between mitochondria and endoplasmic reticulum (ER) substantially influences mitochondrial Ca^{2+} signaling, contributing to cellular bioenergetics. On the other hand, dysregulation of these events plays important roles in the progression of Alzheimer's disease (AD) neuropathology. Here, we performed RNAseq on two AD knock-in mouse models at different disease stages. These mouse models contain a humanized Aβ region with the Swedish and the Iberian – *App*$^{NL-F}$, plus Arctic – *App*$^{NL-G-F}$, pathogenic mutations. Pathway analysis revealed that at pre-symptomatic stages both models display low Ca^{2+} signaling (FDR=0.01) concomitantly with highly upregulated oxidative phosphorylation (FDR<0.01), with the expression of genes belonging to mitochondrial complex I, IV and V mainly affected. Remarkably, embryonic neuronal cultures derived from *App*$^{NL-F}$ mice exhibit increased mitochondrial respiration and ATP production ($p<0.05$), while presenting lower capacity to handle Ca^{2+} release from the ER ($p<0.05$). Electron micrographs of these cultures also revealed increased mitochondria-ER contact sites (MERCS) ($p<0.05$), accompanied by lower levels of Mfn2, a tethering antagonist, partially explaining these early alterations. With pathology progression, Ca^{2+} signaling-related genes such as ER Ca^{2+} channels inositol 1,4,5-trisphosphate receptor type 3 (IP_3R3) and SERCA Ca^{2+}-ATPase

paralogs, show a gradual upregulation (1.85-fold increase for IP_3R3 (FDR<0.0001), and 1.95- and 1.44-fold increase for SERCA1 (FDR=0.09) and 3 (FDR=0.01), respectively), which inversely correlates with decreased expression of electron transport chain genes. These findings suggest that Ca^{2+}-dependent energy dysregulation may be an initial step in the pathogenic cascade leading to AD; therefore, therapeutic interventions targeting bioenergetics may offer a great opportunity for disease-modifying strategies. Taking that into consideration we performed a cell-based high throughput screening where, out of 1,200 FDA-approved compounds, the flavonoid luteolin was identified as a mitochondrial enhancer. Further analysis indicated that under physiological conditions luteolin increases MERCS number *per* mitochondria profile ($p<0.05$) rather than mitochondrial biogenesis or transcription, leading to improved mitochondrial Ca^{2+} homeostasis and ATP production ($p<0.01$). Together, our data suggest that while an initial boost of mitochondrial function may work as a compensatory mechanism in *App* knock-in mice, stabilizers of neuronal Ca^{2+} signaling and mitochondrial function likely have a therapeutic potential in AD and similar neurodegenerative disorders.

Keywords: Alzheimer's disease, mitochondrial Ca^{2+}, oxidative phosphorylation, MERCS, luteolin

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OC3: Inhibition of Sarco-Endoplasmic Reticulum Ca2+ ATPase extends the lifespan in C. elegans worms

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The Sarco-Endoplasmic Reticulum Ca^{2+}-ATPase (SERCA) refills the endoplasmic reticulum (ER) with Ca^{2+} up to the millimolar range and is therefore the main controller of the ER $[Ca^{2+}]$ level ($[Ca^{2+}]_{ER}$), which has a key role in the modulation of cytosolic Ca^{2+} signaling and ER-mitochondria Ca^{2+} transfer. Given that both cytosolic and mitochondrial Ca^{2+} dynamics strongly interplay with energy metabolism and nutrient-sensitive pathways, both of them involved in the aging process, we have studied the effect of SERCA inhibitors on lifespan in *C. elegans*. We have used thapsigargin and 2,5-Di-tert-butylhydroquinone (2,5-BHQ) as SERCA inhibitors, and the inactive analog 2,6-Di-tert-butylhydroquinone (2,6-BHQ) as a control for 2,5-BHQ. Every drug was administered to the worms either directly in the agar or via an inclusion compound with γ-cyclodextrin. The results show that 2,6-BHQ produced a small but significant increase in survival, perhaps because of its antioxidant properties. However, 2,5-BHQ produced in all the conditions a much higher increase in lifespan, and the potent and specific SERCA inhibitor thapsigargin also extended the lifespan. The effects of 2,5-BHQ and thapsigargin had a bell-shaped concentration dependence, with a maximum effect at a certain dose and smaller or even toxic effects at higher concentrations. Our data show therefore that submaximal inhibition of SERCA pumps has a pro-longevity effect, suggesting that Ca^{2+} signaling plays an important role in the aging process and that it could be a promising novel target pathway to act on aging. Insights on the mechanism of the effect have been obtained by using mutants of several key aging pathways and the results suggest an involvement of mitochondria and the AMP kinase and TOR pathways.

Keywords: SERCA, thapsigargin, Ca^{2+}, C. elegans, lifespan

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OC4: Extra-synaptic NMDA receptors, β-amyloids and neuronal activity

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Amyloids-b oligomers are known to alter neuronal signalling through various mechanisms involving NMDA receptors and dysregulation of calcium homeostasis. In this study, we use a computational modelling approach, closely based on experimental data, to investigate the impact of these perturbations on neuronal electrical activity. Since it is known that b-amyloids oligomers increase the activity of extrasynaptic NMDA receptors, we take these channels into account in a computational description of the dynamics of voltage and calcium concentration during neuronal electrical activity. We find that calcium influx through these NMDA receptors can directly be coupled to activation of calcium-activated potassium channels, providing drastic changes in neuronal activity. In particular, it allows the appearance of hysteresis in response to electrical stimuli. Thus, when activated by a ramp in current stimulus, neurons with tonically active NMDA receptors require larger stimuli to become electrically active. On the other hand, when stimulated by a pulse of electrical current, disturbed neurons become locked in a state of long-lasting electrical activity. This hysteretic behaviour may explain the disturbances in electrical activities observed in neurons exhibiting tonic activity of NMDA receptors caused by b-amyloids.

Keywords: mathematical model, neuronal activity, Alzheimer's disease, calcium signalling

OC5: Activation of TRPC1 channel by mGluR5 modulates synaptic plasticity, spatial working memory and memory extinction

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Group I metabotropic glutamate receptors, in particular mGluR5, have been implicated in various forms of synaptic plasticity that are believed to underlie declarative memory. We observed that mGluR5 specifically activated a channel containing TRPC1, an isoform of the canonical family of Transient Receptor Potential channels highly expressed in CA1-3 regions of the hippocampus. We used a mouse model devoid of TRPC1 expression to investigate the involvement of mGluR5-TRPC1 pathway in synaptic plasticity and memory formation. *Trpc1*$^{-/-}$ mice showed alterations in spatial working memory but not in reference memory. Memory extinction was also impaired. Activation of mGluR increased synaptic excitability in neurons from WT but not from *Trpc1*$^{-/-}$ mice. LTP triggered by a theta burst could not maintain over time in brain slices from *Trpc1*$^{-/-}$ mice. mGluR5-induced LTD was also impaired in these mice. Finally, acute inhibition of TRPC1 by Pico145 on isolated neurons or on brain slices mimicked the genetic depletion of *Trpc1* and inhibited mGluR-induced entry of cations and subsequent effects on synaptic plasticity. Memory alterations were confirmed after acute deletion of *Trpc1* obtained by treating adult *Trpc1*$^{lox/-}$ *CamKII-CreERT2* mice with tamoxifen, excluding developmental or compensatory mechanisms in *Trpc1*$^{-/-}$ mice. In summary, our results indicate that TRPC1 plays a role in synaptic plasticity and spatial working memory processes.

Keywords: TRPC channels, mGluR, synaptic plasticity, LTP, LTD

SESSION 2

OC6: *Store-operated calcium channels control neural stem cell self-renewal in the adult brain subventricular zone*

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A tight control of normal function of neural stem cells is thought to be dependent on their microenvironement. One universal way to transduce a signal from the microenvironment is to promote cytosolic Ca^{2+} signals through various receptors including those linked to activation of PLC, which induces calcium release from endoplasmic reticulum and regulates membrane Ca^{2+} channels such as store-operated Ca^{2+} channels (SOC).

In the subventricular zone (SVZ) of the adult mammalian brain, which is a major neurogenic niche where neural stem cells proliferate, self-renew and give birth to neurons and glial cells, brain sections immunostaining revealed that TRPC1, Orai1 and STIM1 are expressed *in vivo,* in SOX2-positive SVZ neural stem cells. The expression in SOX2-positive SVZ cells of these molecular components known to build-up SOC suggested they could be major actors of calcium signaling in stem cells.

In cells isolated from the SVZ and grown in conditions maintaining stemness, we demonstrated that SVZ cells still expressed the major SOC components. Moreover calcium imaging studies revealed that SVZ cells display store-operated calcium entries, in addition to transient calcium signals in response to acetylcholine or HGF and also spontaneous intracellular calcium oscillations. Pharmacological blockade of SOC with SKF 96365, 2 APB or YM 48483 (BTP2), inhibited intracellular calcium oscillations and decreased SVZ cell proliferation. Furthermore, treatment with either SKF 96365 or YM

48483 impaired SVZ neural stem cell self-renewal and affected SVZ cell division by favoring asymmetric cell division at the expense of symmetric proliferative division. Injection of SKF 96365 in brain cerebral ventricles reduced the ability of SVZ cells to form neurospheres *in vitro*, indicating that SOC regulate neural stem cell population. The present study unravels a major role for store-operated calcium channels in SVZ neural stem cells growth and self-renewal.

Keywords: neural stem cell; brain; subventricular; store-operated calcium entry; self-renewal

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OC7: Role of the purinergic P2X7 receptor on intracellular calcium signaling in peripheral blood mononuclear cells of patients with Major depressive disorder

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Major depressive disorder (MDD) is one of the most prevalent among other depressive manifestations and mood disorders. One of the emerging theories regarding the onset of MDD, points to the involvement of the inflammatory processes where purinergic signaling plays an important role.

The P2X receptor family is comprised of ligand-gated ion channels that respond to extracellular adenosine triphosphate (ATP) and increases permeability of calcium (Ca^{2+}) into the cell. The underlying mechanisms of purinergic signalling on peripheral blood mononuclear cells (PBMCs) in MDD is unclear. Herein, we studied the functionality of P2X7 receptors and Ca^{2+} homeostasis in blood cells of MDD patients. In the present work we attempt to make a correlation between P2X7 activation, and its influence on Ca^{2+} homeostasis and the inflammatory stage of the MDD patients. By performing live Ca^{2+} imaging experiments, we show that, ATP (100 μM) stimulation caused a sustained decrease in $[Ca^{2+}]_c$ levels in the MDD group versus healthy controls, as measured by the area under the curve and peak height. Western blot analysis revealed that inflammasome markers such as caspase-1 was increased in MDD patients versus healthy controls. To further understand the role of P2X7R, we performed pharmacological modulation of

P2X7 receptors with the drug JNJ-47965567 (JNJ). To this end, upon JNJ (100 nM) perfusion, PBMCs were stimulated with BzATP (250 mM). We found that application of JNJ decreased Ca^{2+} leves in PBMCs from healthy controls.

Our results indicate an alteration of the functionality of purinergic receptors on PBMCs from MDD patients, leading to intracellular $[Ca^{2+}]_c$ dysregulation. Pharmacological modulation of P2X7R with the compound JNJ could serve as a new therapeutic approach against the inflammatory signaling pathway triggered by P2X7R.

Keywords: Calcium, Purinergic receptors, P2X7, *Major depressive disorder*

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OC8: Effect of Hereditary Spastic Paraplegia genes on presynaptic calcium handling

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Genes that code for ER-shaping proteins are among the most commonly mutated in Hereditary Spastic Paraplegia (HSP). Mutation of these genes in model organisms can lead to disruption of the ER network. To investigate how the role of the ER in calcium signalling is affected by this disruption requires tools to interrogate this. We have developed GAL4-driven genetically encoded Ca^{2+} indicators targeted to the ER lumen, to record Ca^{2+} fluxes in identified *Drosophila* neurons. Using *GAL4* lines specific for Type 1b or Type 1s motor neurons, we compared the responses of different lumenal indicators to electrical stimulation, in axons and presynaptic terminals. The most effective sensor had a Ca^{2+} affinity close to the predicted ER lumenal concentration, and in general showed transiently increased lumenal Ca^{2+} on nerve stimulation. We describe the wildtype ER lumenal response to electrical stimulation, and how this is affected in *Drosophila* mutants for reticulon and REEP genes.

Keywords: endoplasmic reticulum, neurodegeneration, HSP

OC9: Mesmerize: A powerful highly versatile, expandable, and self-documenting platform for calcium imaging analysis

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Calcium imaging data analysis in neurobiology and other fields requires a traceable, efficient and expandable platform that is accessible to both programmers and non-programmers. We present *Mesmerize,* a calcium imaging analysis platform that encapsulates the entire analysis process, from raw data to interactive e-figures for publication. *Mesmerize* provides a graphical interface to the latest analysis methods from the CaImAn library for pre-processing, and signal extraction. *Mesmerize* is highly modular and written in Python, providing access to an immense ecosystem of analysis libraries. It allows for customizable annotation of experiment-specific data that integrate into a simple yet expandable data structure. Ultimately, this allows for interactive figures where the raw data and entire analysis procedures are readily displayed by clicking on the data points in a figure. This will greatly improve reproducibility and facilitate the sharing of raw data. We demonstrate how *Mesmerize* can be applied to investigate a broad range of scientific questions by using datasets ranging from the mouse barrel cortex, the epidermis, notochord and migratory mesenchymal cells of the basal chordate *Ciona intestinalis*, and its neuronal and glial cell populations in the brain. We also show live-demos with e-figures from these datasets.

Mesmerize introduces two analysis tools that are novel in the field of calcium imaging analysis, k-Shape clustering and the Wasserstein distance, to quantitatively describe unique calcium activity patterns. By using spontaneous imaging data from the *Ciona* brain*,* we demonstrate how the Wasserstein distance is a powerful tool for quantitative categorization of disparate cell types based solely on their activity profiles. K-Shape clustering is a technique created by John Paparrizos and Luis Gravano for clustering of time-series data. We show how this technique can be used to quantitatively categorize events, such as peaks or behavioral periods, based on the shape of activity patterns.

These events, which are like letters of an alphabet, can be used to describe calcium dynamics as sequences of discrete letters to find unique or stereotyped sequences. Put together, k-Shape and the Wasserstein distance allow us to create and approach new questions with a greater level of quantitative depth.

Official Mesmerize website: mesmerizelab.org

Keywords: analysis, software, Python, whole-brain, Ciona

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OC10: Shedding light on intracellular organelle contacts with novel modular genetically encoded sensors

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In the last decades, the study of functionally specialized organelles has increased in complexity due to the finding that virtually, every delimiting membrane within the cell can be closely apposed and tethered in a morpho-functional hug that coordinate essential physiological processes. The existence of membrane contact sites (MCSs) introduced the concept that an orchestrated network of specialized membranes co-evolved to perform a novel function. The largest organelle in the cell, the endoplasmic reticulum (ER), forms MCSs with mitochondria, Golgi, endosomes, peroxisomes, lipid droplets and with the plasma membrane (PM). How and at which extent these subcellular communication membrane platforms mediate and regulate cell homeostasis remains obscure. The latter is of particular relevance since MCSs' deregulation is linked to several pathological conditions such as neurodegenerative diseases. To date, the lack of tools to image inter-organelle proximity over a range of distances hindered the possibility to explore their behavior under physiologic conditions and, importantly, in living organisms. We have designed modular, split-GFP based contact site sensors (SPLICS) engineered to fluoresce when narrow (10nm) and wide (50nm) juxtapositions between organelles are present. We have documented the existence of functionally different contacts (e.g. ER-mitochondria) in cellulo and in vivo. SPLICS sensors were improved an extended by creating a single vector with equimolar expression of the GFP fragments (SPLICS-P2A) and color

shifted variants to assess novel, different organelle contacts. SPLICS allowed to explore the dynamic nature of the ER-mitochondria interface in the axon of zebrafish sensory neurons and in dendrites and soma of rat primary hippocampal neurons. Thanks to these new-generation sensors the mechanisms and the dynamic nature of organelle contact sites can be explored in health and disease.

Keywords: Calcium, mitochondria, Neurodegenerative diseases, organelle contact sites, endoplasmic reticulum

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POSTERS COMMUNICATION ABSTRACTS

P1: Point mutations in neuronal Ca^{2+}-sensor protein GCAP1 cause disturbances in Ca^{2+}-homeostasis and lead to human retinal degeneration

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Background: GCAP1 is a neuronal Ca^{2+}-sensor protein, which regulates the photoreceptor specific guanylate cyclase 1 (GC-E; gene *GUCY2D*) in a Ca^{2+}/Mg^{2+}-dependent manner. More than twenty point mutations in GCAP1 lead to blindness by causing cone/rod dystrophies and retinal degenerative diseases. Recently, some of us reported a point mutation G86R in GCAP1 that exhibited prototypical biochemical properties causing dominant retinopathy (Peshenko et al., 2019, JBC 294, 3476 pp). A tryptophan at position 94 (W94) seems to be critical for the conformational change in GCAP1 switching from the activator to the inhibitor state. Aim of present work is to test the function of W94 in combination with the disease causing amino acid exchange G86R.

Methods: Human wildtype and mutant GCAP1 forms (G86R/W94F and G86R/W94L) were expressed and purified from *E.coli* extracts. All GCAP1 variants were tested for Ca^{2+} and Mg^{2+} binding by isothermal titration calorimetry (ITC). Target guanylate cyclase, human GC-E was expressed in HEK293 cells and incubated with purified GCAP1 forms. Enzyme activity was tested as a function of free Ca^{2+}-concentration. Tryptophan fluorescence was used to test protein conformational changes in conditions of high and low Ca^{2+} and protein dimerization was monitored by analytical SEC. Molecular dynamics simulations were applied to interpret our biochemical results in a structural framework.

Results: According to our ITC data the double G86R/W94L showed stronger Ca^{2+} binding than G86R/W94F, the same pattern was observed when the protein bound Mg^{2+}. Mg^{2+} binding of G86/W94L was compromised as compared to G86R single mutant. In case of G86R/W94F the Ca^{2+}-sensitive regulation of the cyclase shifted to higher free Ca^{2+}-concentration keeping the GC/GCAP1 complex constitutively active as seen in G86R. Surprisingly, G86R/W94L was much less effective in activating GC-E at low [Ca^{2+}] indicating that the protein was hindered to fully switch to its activator state. Tryptophan fluorescence measurements showed that G86R/W94L exhibited WT like properties, but mutant G86R/W94F did not. Analytical SEC data indicates a higher tendency of forming dimers in case of G86R/W94F.

Conclusion: Our results support a critical role of residue W94 in the control of conformational switches in GCAP1 that are severely affected in disease causing mutations.

Keywords: retinal degeneration, Ca^{2+} sensor proteins, conformational switches

P2: Mitochondrial Ca^{2+} dynamics in MCU knockout C. elegans worms

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The mitochondrial [Ca2+] plays a very important role in the regulation of mitochondrial function, controlling energy supply to the respiratory chain, ATP production, mitochondrial membrane potential and apoptosis triggered by mitochondrial Ca^{2+} overload. This regulation depends on Ca^{2+} entry to the mitochondria during cell activation processes, which is thought to occur through the mitochondrial Ca^{2+} uniporter (MCU), the main Ca^{2+} entry pathway into the mitochondria. However, knockout mice of the MCU protein, the main component of the uniporter complex, showed surprisingly little effects in the phenotype, in spite of the large blockade of mitochondrial Ca^{2+} uptake. We have studied here the mitochondrial Ca^{2+} dynamics in control and MCU-defective C. elegans worms in vivo, by using worms expressing mitochondrially-targeted YC3.60 yellow cameleon in the pharynx muscle. Confocal images showed a typical mitochondrial distribution of the Ca^{2+} sensor. Our data show that the small mitochondrial Ca2+ oscillations induced by neuronal stimulation with serotonin were very similar in both control and MCU-defective worms. However, direct pharynx muscle stimulation with carbachol triggered a large and prolonged increase in mitochondrial [Ca^{2+}] that was much larger in control worms than in MCU-defective worms. This suggests that MCU is necessary for the fast mitochondrial Ca^{2+} uptake induced by large cell stimulations. However, low amplitude mitochondrial Ca^{2+} oscillations occurring under more physiological conditions are independent of MCU and use a different pathway.

Keywords: Mitochondria, Ca^{2+}, MCU, C. elegans, yellow cameleon

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P3: Calmodulin inhibition of IP3 receptor mediated calcium release: implications of disease-causing calmodulin mutations

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Inositol 1,4,5-trisphosphate (IP3) receptors (IP3Rs) function as gate-keepers for calcium (Ca^{2+}) release from internal stores into the cytosol in a variety of cell types, including neurons. Accordingly, abnormal Ca^{2+} signaling through IP3Rs have been associated with human brain pathology. IP3Rs are regulated not only by IP3, but also by Ca^{2+} itself. While the site triggering IP3R opening is believed to be situated in the receptor itself, the site triggering Ca^{2+} mediated channel closing remains uncharacterized, and this process may be indirectly controlled through an accessory protein. The Ca^{2+}-binding protein calmodulin (CaM) is well-known for its ability to modulate Ca^{2+}-transporting proteins, such as plasma membrane Ca^{2+}-channels and sarcoplasmic reticulum ryanodine receptors. Indeed, CaM has also long been known to exert an inhibitory effect on IP3R Ca^{2+} release. Nevertheless, details about the overall regulation mechanism remain elusive. Curiously, CaM has been reported to modulate IP3Rs both in a Ca^{2+}-dependent and -independent manner. Individuals who carry a mutation in CaM appear to present primarily with a cardiac-specific phenotype. This is surprising given the similarly critical role of CaM in other tissues and organs, such as the brain. Hence, due to the acuteness of the cardiac dysfunction in CaM mutation patients, concomitant deleterious effects, e.g. in the brain, may have been overlooked or may not become apparent until later in life. As the IP3Rs constitute a possible link between neurological disease and CaM dysregulation, we here investigated the Ca^{2+}- and IP3-dependence of CaM regulation of IP3R1. Moreover, we assessed the influence of engineered as well as human disease-causing CaM

mutations on IP3R1 calcium release. Our results suggest a possible functional consequence of CaM mutations due to abnormal modulation of IP3Rs, which we speculate could involve a pathological neuronal susceptibility.

Keywords: IP3 receptor, calmodulin, calmodulin mutations

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P4: PINK1 and PKA interplay in the control of mitochondrial Ca^{2+} homeostasis

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Mutations in the mitochondrial serine–threonine kinase PINK1 are associated with familial forms of Parkinson's disease and mitochondrial Ca^{2+} overload (1,2). The targeting of PKA to mitochondria and its activation rescue functional defects observed in PINK1 deficient neurons (3) and mitochondrial Ca^{2+} overload due to the loss of PINK1 function (4). PINK1 and PKA have been proposed to cooperate at the mitochondria level to prevent neurodegeneration, and we have found that PINK1 was able to reduce mitochondrial Ca^{2+} accumulation. Sustained Ca^{2+} accumulation into the mitochondrial matrix has been shown to correlate with increases of cAMP levels in the same compartment (5). If the localization and the action of PKA at the outer mitochondrial membrane (OMM) are well recognized, its presence in the mitochondrial matrix and in the intramembrane space (IMS) is still amply debated.

We developed a probe based on the split-GFP protein and Bimolecular Fluorescence Complementation (BiFC) to monitor PKA distribution at sub-mitochondrial level in living cells. The non-fluorescent GFP_{1-10} fragment was targeted to the OMM, the IMS and the mitochondrial matrix by the addition of targeting sequences. The β_{11} fragment, necessary to reconstitute GFP fluorescence, was fused to two PKA regulatory subunits (RIα and RIIβ) and to PKA catalytic subunit (Cat-α).

The co-transfection of the plasmids encoding the targeted GFP_{1-10} fragments and the Cat-α β11 or the RIα β11or the RIIβ β11 in Hela cells revealed the presence of all these subunits at the OMM and IMS. Interestingly, strong GFP fluorescence emission in the presence of GFP_{1-10} fragment targeted to mitochondrial matrix was observed in the case of Cat-α β11 co-expression, but not of RIα β11and RIIβ β11, suggesting the presence of PKA Cat-α in the mitochondrial matrix. To evaluate whether regulatory subunits could interfere with mitochondrial Cat-α localization, we co-transfected the GFP1-10 fragment targeted to OMM, IMS and mitochondrial matrix with Cat-α β11 and RIα or RIIβ without β11 fragment. In these conditions we still observed fluorescence reconstitution at the OMM and IMS, but not in the mitochondrial matrix when Cat-α β11 was co-expressed together with RIIβ, suggesting that Cat-α may translocate to this compartment only upon activation and release from its regulatory subunit. Further investigations are necessary to reveal the mechanism and the cellular conditions that favour Cat-α distribution at mitochondrial level and eventually its interplay with mitochondrial PINK1 kinase.

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Keywords: Parkinson's disease, mitochondria, calcium, PINK1, PKA

P5: Amyloid-β-induced neuronal hyperactivity reduces brain fitness and exacerbates neurodegeneration in *Drosophila*

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The major pathological hallmarks of Alzheimer's disease (AD) are neuronal loss and synaptic dysfunction. Excessive accumulation of the toxic peptide amyloid-ß is considered the trigger of the disease but the subsequent cascade of events is not completely understood yet. Using a *Drosophila* model that expresses a human amyloid-β42 transgene and recapitulates key features of AD, I recently reported that removal of less fit neurons via fitness comparison has a beneficial effect, protecting against brain degeneration, motor decline and memory loss.

To characterize neurons eliminated by fitness comparison, I employed genetically encoded Ca^{2+} reporters that respond to neuronal activity and monitored fitness markers in amyloid-β transgenic flies. I observed that ectopic amyloid-β causes neuronal hyperactivation and is associated with an increased accumulation of the excitatory neurotransmitter glutamate in the *Drosophila* brain. Sustained activation of neurons with the thermo-inducible cation channel TRPA1 was sufficient to decrease neuronal fitness and to cause apoptosis. Moreover forced silencing of neurons by ectopic expression of a K^+ channel in amyloid-β-flies rescued neuronal fitness and decreased apoptosis in the brain of these flies.

These preliminary results suggest that less fit neurons which are eliminated by fitness comparison surprisingly do not correspond to nonfunctional or silenced neurons, but rather to hyperactive neurons which may disturb neural network communication and compromise cognitive performance.

Keywords: Neuronal fitness, Drosophila, Alzheimer's, Apoptosis, Neurodegeneration, Neural hyperactivation, Cell competition, Calcium

P6: Astrocytic Ca^{2+} dynamics and gliotransmitter release in Alzheimer's disease conditions

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Alzheimer's disease (AD) is characterized by progressive memory decline associated with synaptic dysfunction and loss caused by amyloid-β peptides (Aβ) accumulation. Astrocytes are able to control synaptic function, exhibiting transient oscillations in intracellular Ca^{2+} levels that are thought to mediate gliotransmitter release (e.g. ATP and glutamate) which have modulatory actions on other astrocytes and neurons. Our group showed that astrocytic adenosine A_{2A} receptors ($A_{2A}R$) control glutamate clearance, a process crucial for synaptic transmission, which is reduced after Aβ exposure. However, it remains to be clarified whether Aβ affects Ca^{2+} dynamics and gliotransmission and if these mechanisms are modulated by $A_{2A}R$.

This study aims to investigate: i) how Aβ exposure affects astrocytic Ca^{2+} levels and Ca^{2+} -dependent gliotransmitter release, and ii) if $A_{2A}R$ modulate Ca^{2+} dynamics and gliotransmission. Therefore, we used primary cultures of rat astrocytes exposed to $A\beta_{1-42}$ (1μM, 1h) and monitored astrocytic Ca^{2+} levels by fluorescence live-cell imaging; the extracellular levels of ATP and glutamate were also quantified.

Astrocytes exposed to $A\beta_{1-42}$ showed a significant increase in the magnitude of ATP-evoked Ca^{2+} response ($p<0.05$), with a lower amplitude and a longer duration (more 130 sec) of Ca^{2+} response. The astrocytic ATP-evoked Ca^{2+} response was significantly reduced ($p<0.01$, about 86% decrease) by the Ca^{2+} chelator EGTA (50 μM), and also by Xestospongin C (1 μM), an inhibitor of IP_3 receptors of endoplasmic reticulum (ER), indicating that extracellular Ca^{2+} influx and Ca^{2+} release from ER contributed to alterations in ATP-evoked Ca^{2+} response in AD conditions. The selective antagonist of $A_{2A}R$, SCH58261,

decreased the magnitude of ATP-evoked Ca^{2+} response ($p=0.07$) in $A\beta_{1-42}$-treated astrocytes, suggesting that $A_{2A}R$ control astrocytic Ca^{2+} dynamics. Furthermore, we observed that the intracellular (BAPTA-AM) and extracellular (EGTA) Ca^{2+} chelators significantly ($p<0.001$), prevented the increased ATP release observed in $A\beta_{1-42}$-treated astrocytes suggesting that Ca^{2+}-dependent ATP release was affected in AD conditions. $A\beta_{1-42}$ also increased extracellular glutamate levels, however BAPTA-AM and EGTA had no significant effects.

Overall, these results showed that $A\beta_{1-42}$ exposure affected Ca^{2+} dynamics and likely exocytotic ATP release, and $A_{2A}R$ blockade prevents these alterations. Thus, astrocytic $A_{2A}R$ seem to be a valuable therapeutic target in AD.

Keywords: Ca2+ dynamics, live-cell imaging, exocytotic gliotransmitters release, astrocytes, Alzheimer's disease

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P7: Metabolic consequences of altered ER to mitochondria Ca^{2+} transfer in FAD-Presenilin 2 models.

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In neurons, mitochondria are essential to produce ATP and sustain normal synaptic activity, which involves a multitude of high energy-demanding processes. Specifically, in the mitochondrial matrix, the Krebs cycle extracts electrons from carbon sources and supplies them to the electron transport chain, sustaining ATP synthesis. Importantly, Ca^{2+} signal modulates the activity of multiple carriers (essential to take up different substrates) and stimulates different Krebs cycle enzymes, playing a key role in the regulation of mitochondrial metabolism. It has been shown that the constitutive inositol trisphosphate (IP3)-mediated Ca^{2+} transfer from the endoplasmic reticulum (ER) to mitochondria modulates cell bioenergetics, by enhancing mitochondrial activity. We previously demonstrated that different Presenilin 2 (PS2)-mutants, linked to familial Alzheimer's disease (FAD), affect ER Ca^{2+} content and ER-mitochondria Ca^{2+} shuttling. Here, we investigate the effects of these Ca^{2+}-signalling alterations on mitochondrial and cell metabolism. We found that, in different FAD-PS2 models, including primary cortical neurons from FAD-PS2-N141I transgenic (tg) mice, mitochondrial ATP synthesis is impaired. The defect depends on a reduced mitochondrial pyruvate oxidation, due to both lower Ca^{2+}-mediated stimulation of the Krebs cycle and dampened mitochondrial pyruvate uptake. We correlated this latter event to glycogen-synthase-kinase-3b (GSK-3b) hyper-activation, leading, in turn, to impaired recruitment of Hexokinase 1 (HK1) to mitochondria and destabilization of the mitochondrial-pyruvate-carrier (MPC)-complexes. Importantly, the defective mitochondrial bioenergetics influences glutamate-induced neuronal excitotoxicity. Potential implications of these alterations for neurodegeneration are discussed.

Keywords: Alzheimer's disease, calcium, mitochondria, bioenergetics, pyruvate

P8: Mitochondrial dysfunctions as an early event in the Pathogenesis of Familial Alzheimer's disease?

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Alzheimer's disease (AD) is the most common form of dementia, in which learning, memory and cognitive function decline simultaneously, dramatically and permanently. Ca^{2+} homeostasis impairment and mitochondrial dysfunction are crucial events associated with several neuropathologies, including AD, ultimately leading to neurodegeneration by still largely unknown mechanisms. Most of the AD cases are sporadic, while only a small percentage are genetic (Familial AD, FAD) and mainly due to mutations in genes encoding presenilin (PS) 1, PS2 and APP (amyloid precursor proteins). These proteins, in addition to their role in β-amyloid plaques formation, directly affect Ca^{2+} signalling.

Here, we aim at uncover the role of Ca^{2+} dyshomeostasis in FAD neuronal dysfunction. Employing a FAD mouse model (PS2-N141I/APPswe) in combination with two-photon time-lapse imaging techniques, we are investigating the effects of FAD mutations on cellular and mitochondrial Ca^{2+} dynamics in hippocampal slices at different ages, exploiting the intracranial injection of adeno-associated virus expressing FRET-based Ca^{2+} probes. Intracellular Ca^{2+} rises induced by ionotropic (NMDA) or metabotropic (DHPG) glutamate receptor stimulation were similar in their peaks in FAD and WT mice at 1.5 month of age, although the duration of the Ca^{2+} increases were substantially longer in FAD mice. The mitochondrial Ca^{2+} uptake ability, in response to the same stimulations, is currently under investigation.

To molecularly characterize Ca^{2+} signal dysfunctions, and their correlation with neuronal death, we exploit RT-qPCR analysis of cortices and hippocampi derived from FAD and WT mice, at different age during disease progression. An upregulation of the Mitochondrial Calcium Uniporter (MCU), and of its

several regulatory subunits, has been found in FAD mice, compared to WT controls, at 1.5 month of age. Furthermore, at this age, FAD mice, compared to WT, showed upregulation and downregulation of several genes involved in both inhibitory and excitatory neurotransmission; similar results were obtained concerning genes linked to autophagy, mitophagy, apoptosis and necrosis. Altogether, these results suggest that a substantial rearrangement of gene expression occur early in FAD mice, especially of those involved in Ca^{2+} homeostasis and cell death control. These latter may potentially represent novel therapeutic targets towards the treatment of AD.

P9: The neuroprotector benzothiazepine CGP37157 extends lifespan in C. elegans worms

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The benzothiazepine CGP37157 has shown neuroprotective effects in several *in vitro* models of excitotoxicity involving dysregulation of intracellular Ca^{2+} homeostasis. Although its mechanism of neuroprotection is unclear, it is probably related with some of its effects on Ca^{2+} homeostasis. CGP37157 is a well-known inhibitor of the mitochondrial Na^+/Ca^{2+} exchanger. However, it is not very specific and also blocks several other Ca^{2+} channels and transporters, including voltage-gated Ca^{2+} channels, plasma membrane Na^+/Ca^{2+} exchanger and the Ca^{2+} homeostasis modulator 1 channel (CALHM1). In the present work, we have studied if CGP37157 could also induce changes in life expectancy. We now report that CGP37157 extends *C. elegans* lifespan by 10-15% with a bell-shaped concentration–response, with high concentrations producing no effect. The effect was even larger (25% increase in life expectancy) in worms fed with heat-inactivated bacteria. The worm CGP37157 concentration producing maximum effect was measured by HPLC-MS/MS and was close to the IC_{50} for inhibition of the Na^+/Ca^{2+} exchanger. CGP37157 also extended the lifespan in *eat-2* mutants (a model for caloric restriction), suggesting that caloric restriction is not involved in the mechanism of lifespan extension. Actually, CGP37157 produced no effect in mutants of the TOR pathway (*daf15/unc24*) or the insulin/IGF-1 pathway (*daf-2*), indicating that

the effect involves these pathways. Moreover, CGP37157 was also ineffective in *nuo-6* mutants, which have a defect in the mitochondrial respiratory chain complex I. Since it has been described that neuroprotection by this compound in cell cultures is abolished by mitochondrial inhibitors, this suggests that life extension in C. elegans and neuroprotection in cell cultures may share a similar mechanism involving mitochondria.

Keywords: CGP37157, Ca^{2+}, C. elegans, lifespan, neuroprotection

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P10: Calcium signalling and mitochondrial function in Presenilin 2-knock-out mice: is there any loss-of-function phenotype related to Alzheimer's disease?

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Alzheimer's disease (AD) is the most common form of dementia characterized by progressive cognitive dysfunctions. The histopathological hallmarks of AD brains are extracellular neuritic plaques, formed by amyloid beta (Aβ) peptides and intracellular neurofibrillary tangles of hyper-phosphorylated tau. Familial cases of AD (FAD) are due to mutations in one of three genes encoding amyloid precursor protein (APP), presenilin 1 (PS1) and presenilin 2 (PS2). PS1 and its homologue PS2 form the catalytic core of the γ-secretase complex that, by cleaving APP in concert with β-secretase, produces neurotoxic Aβ peptides. Recently, FAD-PS1 mutations have been associated with a loss-of function phenotype for γ-secretase activity, since a FAD-resembling phenotype was described in mice knock-out (KO) for PS1.

With this project, we intend to verify if the model propose for FAD-PS1 mutants can be extended also to PS2.

In the last years, evidence has accumulated suggesting that AD is linked to an imbalance of cellular Ca^{2+} homeostasis. In particular, FAD-PS2 mutations cause a reduction in the endoplasmic reticulum (ER) Ca^{2+} content, by inhibiting SERCA activity, while increase the physical apposition between ER and mitochondria, favouring ER to mitochondria Ca^{2+} transfer and modulating organelle activity. We thus investigated Ca^{2+} signalling and mitochondrial function in cortical and hippocampal neurons from PS2-KO mice to check whether the previous characterized FAD-PS2-linked phenotypes could be considered loss-of function. For Ca^{2+} measurements, cortical and hippocampal PS2-KO neurons (and control cells from wild type, WT, animals) have been

infected in vitro by Aden-Associated viruses expressing the cDNA of FRET-based cytosolic or mitochondria-targeted Ca2+ probes. Neurons were stimulated with a mixture of metabotropic or depolarizing stimuli. Despite similar cytosolic Ca^{2+} rises, PS2-KO neurons show a significantly reduced mitochondrial Ca^{2+} uptake, compared to those observed in WT neurons, indicating that the loss of PS2 does not recapitulate FAD-PS2-linked mitochondrial phenotype. The general impairment in mitochondrial Ca^{2+} handling is currently under investigation to understand whether it could be considered a primary defect. Preliminary results indicate no difference in mitochondrial number, morphology and protein content. However, a significant reduction in basal, maximal and ATP-linked respiration is present in PS2-KO neurons, compared to WT cells.

Keywords: Familial Alzheimer's disease, Presenilin, mitochondria, FRET, Ca^{2+} imaging.

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P11: A novel mutation in calmodulin causes dysregulation of calcium channels

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Calmodulin (CaM) is expressed in all cells and plays an immense role for maintaining cellular health. Here, CaM regulates a large battery of proteins from ion channels to transcription factors. Mutations in CaM in human patients were discovered within the last 10 years, and, interestingly, all identified patients present with severe cardiac arrhythmia. However, scientists ask whether CaM mutations may have effects in other tissues that heavily rely on CaM regulation – such as neurons – that are yet to be discovered.

Here, we present the identification and characterization of a novel CaM mutation. The mutation was identified in a 5-year old Danish boy with ADHD, who presented with cardiac arrest while driving a moon-car in nursery. Sequencing revealed that the boy had a *de novo* missense-mutation in *CALM1*, resulting in an amino acid G132V substitution in CaM.

Biophysical experiments showed that the calcium binding affinity of the CaM-G132V C-terminal domain was strongly compromised. Thus, in this patient, mutated CaM does not register changes in cytosolic calcium as precisely as non-mutated CaM. This mutation would thus be expected to impair normal channel closure. To address this, we measured CaM regulation of two major calcium channels in the heart: First, we used electrophysiology of HEK293 cells expressing voltage-gated calcium channel 1.2 ($Ca_V1.2$) and CaM-G132V or CaM-WT. We observed a reduction of calcium-dependent inactivation (CDI). Similarly, to observe the effects on closure of the ryanodine receptor 2 (RyR2), we used calcium imaging of HEK293 cells expressing RyR2 and CaM-G132V or CaM-WT to measure sarcoplasmic calcium release. Again, we found that RyR2 closure was strongly impaired.

The identification of this novel CaM mutation carrier adds to an expanding group of individuals with mutations in CaM. In this patient, the G132V substitution caused reduced calcium binding affinity and compromised regulation of RyR2 and $Ca_V1.2$, ultimately leading to arrhythmia. If ADHD is part of the phenotypic consequence of this mutation is unknown. In light of the strong functional effect on $Ca_V1.2$ detected, it is interesting that *CACNA1C* (encoding $Ca_V1.2$) is one of the best established risk genes for psychiatric disease, including ADHD.

Keywords: calmodulin, arrhythmia, calcium binding, de novo mutation, calcium channel regulation

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P12: Histone deacetylase inhibitors prevent mitochondrial calcium retention in hippocampal neurons from a mice model of Alzheimer's disease

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Alzheimer's disease (AD) is the most prevalent age-related neurodegenerative disorder associated with loss of neurons in the hippocampus, progressing to the cortex. Extracellular senile plaques containing amyloid-beta peptide (Abeta) and intracellular neurofibrillary tangles constitute AD pathological hallmarks. Several molecular pathways have been described in AD, including repressed gene transcription, excitotoxicity and mitochondrial dysfunction; however, no effective therapeutics exist. In the present study we analysed the role of class I histone deacetylase inhibitors (HDACi): sodium butyrate (SB), suberoylanilide hydroxamic acid (SAHA) and tacedinaline (Tac), on mitochondrial function in AD hippocampal cells. Selective activation of N-methyl-D-aspartate (NMDA) receptor (NMDAR) containing GluN2B subunit leads to increased mitochondrial Ca^{2+} (mitoCa) retention and intracellular ROS levels along with mitochondrial hyperpolarization in 3xTg-AD, compared with WT hippocampal neurons. Both mouse hippocampal neurons and HT22 cells treated with nontoxic HDACi increased H3 histone acetylation. SB and Tac also prevented HT22 cytotoxic effects exerted by Abeta1-42 oligomers (AbetaO) pre-treatment. Intracellular free Ca^{2+} (Ca^{2+}_i) levels evoked by NMDA/glycine decreased after SB, SAHA and Tac in 3xTg-AD, but not after immediate exposure to AbetaO in hippocampal neurons. However, enhanced mitoCa retention observed in NMDA/gly-stimulated 3xTg-AD neurons remained

unchanged following HDACi treatment. Incubation with SB, SAHA and Tac significantly increased mitochondrial membrane potential (ΔΨm) after NMDAR activation in WT neurons, but only Tac showed this effect following immediate AbetaO exposure. Data reveal the importance of defining the role of HDACs and their inhibitors in AD pathogenesis involving hippocampal glutamatergic synapses.

Keywords: Alzheimer's disease; gene transcription; histone deacetylases; amyloid-beta peptide

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P13: Ageing-induced ultrastructural and functional changes of pulmonary vein sleeve cells: a contributor to atrial fibrillation in later life?

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Atrial fibrillation is the most common sustained cardiac arrhythmia, with age being a significant risk factor. It is established that electrical activity originating from pulmonary vein sleeve cells (PVCs) can initiate atrial fibrillation. Although PVCs are located outside the heart, action potentials that are spontaneously generated by the PVCs can propagate into the atrial chambers and cause unwanted cardiac excitation. It is not entirely clear how PVCs generate these spontaneous action potentials, nor why the prevalence of AF increases with age. Factors that are involved in triggering and maintaining atrial fibrillation include structural remodelling and abnormal Ca^{2+} handling. Whilst structural changes and alterations in Ca^{2+} homeostasis have been described previously for atrial myocytes, similar studies are lacking for PVCs. We investigated ageing-induced changes in the structure and function of PVCs by comparison of myocytes from 3- and 24-month-old mice.

Using immunohistochemical and electron microscopy, we observed that PVCs, just like atrial and ventricular myocytes, express type 2 ryanodine receptors (RyR2), connexin 40 (Cx40), and connexin 43 (Cx43). Whilst neither RyR2 nor Cx43 expression changed with age, Cx40 expression was found to be reduced in the older animals. Additionally, EM studies showed significant alterations in the size, shape and number mitochondria in the older animals. We developed an index for assessing the organisaion of PVCs, and atrial and ventricular cardiomyocytes, based on the alignment of myofilaments in EM

images. Ventricular myocytes showed the highest degree of organisation in young animals, but the least myofilament alignment in older animals. Atrial myocytes and PVCs were not as organsed as ventricular myocytes in young animals, and were not significantly different in older animals. Using Ca^{2+} imaging, we observed that Ca^{2+} homeostasis was altered in PVCs from the older animals. Specifically, PVCs from the aged mice had an increased frequency of spontaneous Ca^{2+} waves, and were less able to follow electrical pacing. The aged animals displayed higher reactive oxygen species levels compared to PVCs from young mice. In summary, we observed a range of structural and functional alterations in PVCs from aged animals that may facilitate the occurrence of arrhythmias.

Keywords: calcium, ageing, cardiac, ultrastructure, pulmonary vein

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P14: Effects of aging and amyloid oligomers on IP3 receptor expression and IP3 dependent calcium release in rat hippocampal neurons

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Aging is the most important risk factor for Alzheimer´s disease (AD). AD is related to excess of amyloid β oligomers (Aβo) that promote neuron cell damage particularly in aged hippocampal neurons. We have reported recently that hippocampal neurons aged in vitro show an important remodelling of intracellular calcium homeostasis that is exacerbated in the presence of Aβo. Remodelling includes enhanced resting cytosolic Ca^{2+} concentration, Ca^{2+} store content and Ca^{2+} release from intracellular stores induced by acetylcholine (ACh) as well as decreased store-operated Ca^{2+} entry (SOCE), a calcium entry pathway involved in memory storage in the hippocampus. However, whether remodelling involves changes in expression and/or activity of IP_3 receptor channels has not been addressed. Accordingly, we used quantitative immunofluorescence to investigate changes in expression of IP_3 receptor isoforms in aging rat hippocampal neurons and the effects of Aβo. In addition, we used caged IP_3 and confocal microscopy to investigate the effects of aging and Aβo on Ca^{2+} release induced by IP_3. We found that aging increases expression of all three IP_3 receptor isoforms (IP_3R1, IP_3R2 and IP_3R3) in rat hippocampal neurons aged in vitro. In contrast, Aβo decreased specifically expression of IP_3R2 in young neurons but not in aged neurons. Furthermore, we found that Ca^{2+} release induced by caged IP_3 was much larger in neurons aged in vitro than in young neurons. Finally, we also observed that Aβo promoted a differential effect in young and aged neurons. Specifically, in young neurons, Aβo decreased Ca^{2+} release induced by caged

IP_3. However, in aged neurons Aβo promoted the opposite effects. In summary, our results suggest that changes in expression and activity of IP_3 receptors may contribute to calcium remodelling and susceptibility to neuron damage in aging and AD.

Keywords: Aging, Alzheimer´s disease, calcium, IP3 receptors, amyloid oligomers.

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P15: STIM1-Cav1.2-ITPR3 axis regulates mitochondrial function and cell survival in SH-SY5Y cells

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STIM1 is a transmembrane protein located at the endoplasmic reticulum (ER), which plays a key role in Ca^{2+} mobilization and signaling, being the main regulator of store-operated Ca^{2+} entry (SOCE), a Ca^{2+} influx pathway regulated by the Ca^{2+} concentration within the ER. In this regard, STIM1 is essential to maintain Ca^{2+} homeostasis required for cell survival, since the dysregulation of Ca^{2+} mobilization and the loss of STIM1 have been linked to several pathologies, including autoimmune and neurodegenerative diseases. It is known that STIM1 inhibits the voltage-operated calcium channel $Ca_V1.2$ by direct binding of STIM1 to the channel and by enhancing the channel internalization from the plasma membrane. Using CRISPR/Cas-mediated gene editing to knockout *STIM1* gene in the neuroblastoma cell line SH-SY5Y, we have observed a significant mitochondrial dysfunction that led to notable cell death during differentiation of these cells because of $Ca_V1.2$ upregulation (Pascual-Caro *et al.*, 2018). On the other hand, STIM1 is a positive regulator of inositol 1,4,5-trisphosphate receptors (IP_3Rs) and IP_3Rs activation enhances STIM1-IP_3Rs association leading to an increased Ca^{2+} influx through SOC channels (Sampieri *et al.*, 2018). Thus, STIM1 and IP_3Rs play an important role in Ca^{2+} shuttling between ER and mitochondria, a key function to maintain an adequate Ca^{2+} homeostasis and mitochondrial function. Our results indicate that STIM1-KO cells show a strong decrease of ITPR3 (or IP_3R3) mRNA and protein expression, as well as a decrease in the mitochondria free Ca^{2+}

concentration ($[Ca^{2+}]_{mito}$), compared to wild-type cells. Finally, the knocking-down of *CACNA1C* gene (encoding for $Ca_V1.2$ channel) restored mitochondrial function and normalized ITPR3 levels in STIM1-KO cells, and the overexpression of the ITPR3 receptor in STIM1-deficient cells contributed to the normalization of $[Ca^{2+}]_{mito}$, suggesting an essential link between STIM1 levels and cell survival mediated by the STIM1-$Ca_V1.2$-ITPR3 axis.

Keywords: Calcium, CRISPR, ITPR3, mitochondria, STIM1

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P16: Role of Endoplasmic Reticulum (ER)-mitochondria Ca^{2+} transfer in NLRP3 inflammasome activation in human monocytes

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Alterations in the innate immune system and impairment of cellular response to endoplasmic reticulum (ER) stress have been associated with aging, which is the major risk factor for several neurodegenerative diseases. Mitochondria-Associated Membranes (MAMs) are ER-mitochondria contacts that regulate inflammatory responses as well as proteostasis, organelles dynamics and mitochondrial metabolism. These lipid raft-like domains allow physical and functional communication between ER and mitochondria, which is crucial for inter-organelle Ca^{2+} and lipids transmission controlling pro-survival/death pathways.

Taking the above evidences in mind, this study aimed to address the role of ER stress in innate immunity and to investigate the role of MAMs, with a special focus on ER-mitochondria Ca^{2+} transfer, on ER stress-induced sterile inflammation. Using a human monocytic cell line (THP-1 cells) treated with classical ER stressors (tunicamycin or thapsigargin), protein levels of markers of the ER stress-induced unfolded protein response (UPR), such as ATF4 and sXBP1, were determined by western blot (WB), and the activation of the NLRP3 inflammasome was analyzed by ELISA. Concomitantly, ER-mitochondria contacts were evaluated by determining the protein levels of several MAM's components by WB. Functional parameters were also investigated under stressful conditions in THP-1 cells, namely ER-mitochondria Ca^{2+} transfer and, mitochondrial Reactive Oxygen Species (ROS) production and membrane potential, using the organelle-specific fluorescent probes Rhod-2 AM, MitoSox and TMRE, respectively.

ER-mitochondria contacts at MAMs were affected in human innate immune cells in response to ER stress, in the absence of mitochondrial ROS accumulation. In these cells, ER stress induced by tunicamycin, but not by thapsigargin, activated the NLRP3 inflammasome. In tunicamycin-treated monocytes, IL1b release occurred upon ER Ca^{2+} release and mitochondrial membrane depolarization, in the absence of mitochondria Ca^{2+} accumulation. On the other side, an increase of mitochondrial Ca^{2+} content was detected in thapsigargin-treated THP-1 cells without alterations in mitochondrial membrane potential.

Overall, our results suggest the establishment of a deleterious axis consisting of ER stress-MAM alterations-inflammasome activation in immune cells, where Ca^{2+} plays a relevant role.

Keywords: Endoplasmic Reticulum (ER) stress, Mitochondria-Associated Membranes (MAMs), ER-mitochondria Ca^{2+} transfer, inflammasome activation, innate immune system

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P17: Effect of phytochemicals on glutamate decarboxylase activity and oxidative stress in PC12 cells with normal and impaired calcium homeostasis

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Disrupted metabolism of neurotransmitters like glutamate or γ-aminobutyric acid (GABA) and impaired balance between their levels (especially excess of glutamate and following excitotoxicity) can lead to development of various neurological, affective or neurodegenerative diseases (e.g. Alzheimer's disease, Parkinson's disease, epilepsy, mood disorders). Additionally glutamate is involved in such processes as synaptic plasticity, learning and memory. Conversion of glutamate to GABA is catalyzed by glutamate decarboxylase (GAD), the enzyme of GABA-shunt. Neurological diseases are also often accompanied by impaired calcium conditions. Drugs commonly used in therapy are not always efficient and often evoke adverse side effects. Naturally occurring in plants compounds, known as strong antioxidants and anticancer agents, are an attractive alternative targets in modulating of neurotransmission without toxic effects. The aim of our study was to investigate an effect of selected phytochemicals: genistein, lycopene and zeaxanthin on GAD activity and oxidative stress in PC12 cell with increased calcium level. We used stabile transfected pseudoneuronal PC12 cells with reduced expression of neuron-specific isoforms of calcium membrane pumps: PMCA2 or PMCA3. Cells were incubated with 1 μM, 5 μM, 10μM and 20μM of tested compounds during 10 and 20 minutes, a short time precluding genomic action or during 6 and 12 hours. Examination of GAD activity is based on fluorometric measurements of product – GABA. Additionally, we used vigabatrin, inhibitor of GABA aminotransferase. Oxidative stress was evaluated as level of H_2O_2 using 2'7'-dichlorofluorescein diacetate which converts into highly fluorescent 2',7'-dichlorofluorescein upon oxidation. Our results indicate that tested compounds increase GAD

activity in control cell lines but inhibit this enzyme in cells with reduced expression of PMCA2 and PMCA3 during 10 or 20 minutes. Prolonged exposition to phytochemicals lessen this inhibitory effect. We conclude that increased calcium concentration abolishes neuroprotective action of genistein, lycopene and zeaxanthin and we suggest non-genomic action of studied phytochemicals due to short time of incubation.

Keywords: glutamate decarboxylase, genistein, lycopene, zeaxanthin, PMCA

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P18: Skin allergens and Alzheimer´s disease: preliminary studies on an (im)probable link

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RATIONALE| Experimental evidences highlight the nuclear factor E2-related factor 2 (Nrf2) as an attractive molecular target in the context of Alzheimer's disease (AD). Electrophilic compounds such as skin allergens are well-known Nrf2 activators and, interestingly, dimethyl fumarate (DMF) was recently approved by FDA and EMA to manage the relapsing forms of multiple sclerosis. Together, these findings led us to the innovative hypothesis that DMF and other unexplored electrophilic molecules could have a preventive and/or therapeutic role in AD.

METHODS| Three drug-like electrophilic chemicals were tested in an *in vitro* AD model, the N2a neuronal mouse cell line overexpressing human wild-type APP (N2a-APPwt) or mutant APP (N2a-APPswe), versus N2a-wt cells, using DMF as a positive control. The expression of the antioxidant Nrf2-dependent gene heme oxygenase (decycling) 1 (*Hmox*1) was analysed by RT-PCR in N2a cells exposed to a non-toxic dose of each chemical (calculated by the resazurin assay and flow cytometry). Protein levels of HMOX1 were evaluated by western blotting, 24 h after electrophile exposure. After 24 h, the intracellular cytosolic ($[Ca^{2+}]i$) and mitochondrial ($[Ca^{2+}]mitoc$) calcium levels were measured using INDO/1-AM and Rhod/2-AM dyes, respectively, and mitochondrial membrane potential was investigated with the TMRE probe.

RESULTS| N2a-APP (wt and mutant) cells expressed lower levels of *Hmox*1 than N2a-wt. The tested electrophilic molecules were able to increase *Hmox*1 gene expression in N2a-APPswe cells, which was detected 3 h (DMF and molecule III) or 6 h (molecule I and II) after chemical exposure. Surprisingly, a two-fold increase in HMOX protein levels was observed in N2a-APPswe compared to N2a-wt cells, suggesting deficient protein degradation. Also, N2a-APPwt cells exhibited higher levels of $[Ca^{2+}]i$ than N2a-wt cells and a rise in $[Ca^{2+}]mitoc$ was observed in both N2a-APPwt and N2a-APPswe. Accordingly, a slight depolarization of mitochondrial membrane was found in APP-overexpressing cells in comparison with N2a-wt. Preliminary results suggest that the tested electrophiles are able to restore Ca^{2+} homeostasis in N2a-APPwt cells as well as to modulate mitochondrial membrane potential in N2a-APP-overexpressing cells.

CONCLUSIONS| The electrophilic molecules tested showed promising results in activating antioxidant defenses mechanisms and preserve mitochondrial function in AD, particularly in an early stage.

Keywords: Alzheimer disease, Nrf2, neurons, electrophiles, allergens

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P19: Pro- and anti-aging effects in differentiated PC12 cells with modified PMCA isoforms composition.

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Among variety of processes triggered during aging, disturbance of neuronal calcium homeostasis appears to be a fundamental one, having far-reaching consequences on cell fate decision. Plasma membrane Ca^{2+}-ATPase (PMCA), the most sensitive calcium detector in cellular Ca^{2+} handling, has been shown to decline gradually during aging. PMCA exists in four main isoforms and PMCA2 and PMCA3 are highly expressed in the brain, thus impaired calcium extrusion in senescent cells can make them more susceptible to neurotoxic insults. To analyze PMCA role in neuronal cells we have developed stable transfected differentiated PC12 lines with down-regulated PMCA2 or PMCA3 isoforms to mimic age-related changes and monitor long-time effects of their suppression. We have observed two types of cell response: similar for both lines or characteristic for only one. In PMCA-modified lines increased cytosolic Ca^{2+} and delayed Ca^{2+} clearance, also affected the expression level of several Ca^{2+}-associated proteins, i.e. SERCA, calmodulin, calcineurin, neuromodulin, CCR5, IP_3 receptors and certain types of voltage-gated calcium channels, however to a different extent. Interestingly, the results revealed triggering of some adaptive processes that were more effective in PMCA3-deficient cells. Functional study has shown that in PMCA2-reduced cells the main source of energy was oxidative phosphorylation, whereas cells with unchanged PMCA2 expression exhibited prevalence of glycolysis in ATP generation. Nonetheless, PMCA2 downregulation increased the number of apoptotic cells. In summary, reduction of PMCA2 appears to be more harmful than suppresion of PMCA3, and adaptive mechanisms aimed to compensate PMCA loss seem

not be sufficient for full cell protection against calcium overload. It could partially explain the progressive decay in physiological function and neuronal death seen over the course of aging.

Keywords: plasma membrane Ca2+-ATPase; calcium homeostasis; neuronal aging; PC12 cells

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