



1st International Conference of
TWAS Young Affiliates Network



RyR-mediated calcium release and mitochondrial ROS generation partake in the synaptic dysfunction caused by amyloid β peptide oligomers

Carol SanMartin^{1, 2}, Pablo Veloso^{3, 1}, Tatiana Adasme^{1, 4}, Alejandra Garcia^{1, 5}, Steffen Hartel^{1, 5}, Cecilia Hidalgo^{1, 6}, Andrea C. Paula-Lima^{3, 1*}



ALZHEIMER'S DISEASE AND SPATIAL MEMORY LOSS



**Early symptom:
Getting lost in familiar spaces**

3 GROUPS:

1. YOUNG CONTROL SUBJECTS (20-40 YEAR-OLD)
2. AGED CONTROL SUBJECTS (OVER 65 YEAR-OLD)
3. EARLY AD PATIENTS (OVER 65 YEAR-OLD)

Duration of each trial: 60 sec

TASK1: HIDDEN PLATFORM

1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4

CONTROL TASK2: VISIBLE PLATFORMS

1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4

- 1) Training: adaptation to the system
- 2) Task I: main memory task
- 3) Task II: control of visual and psychomotor performance

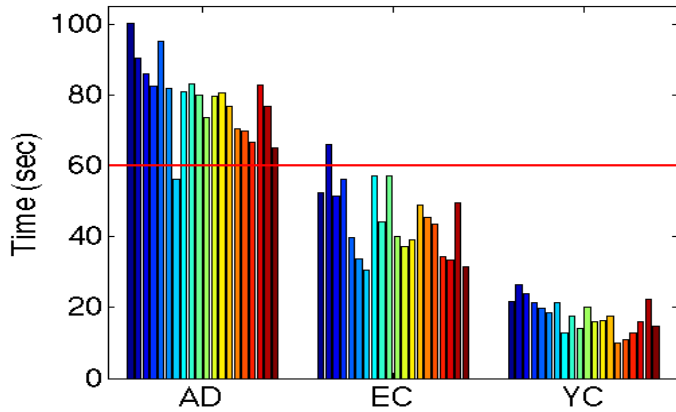
EEG COMBINED TO EYE TRACKING DURING VIRTUAL NAVIGATION



SPATIAL NAVIGATION, VISUAL EXPLORATION AND ELECTRICAL ACTIVITY IS ALTERED IN AD

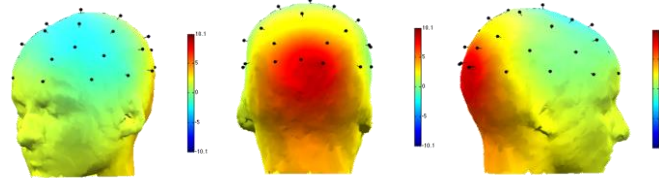


Latency to Platform



Electrical activity associated to eye fixation

60 ms

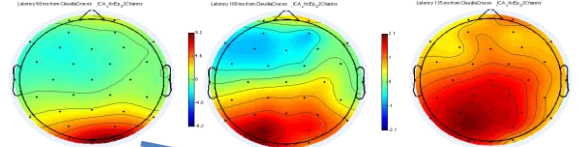


Elderly controls

60 ms

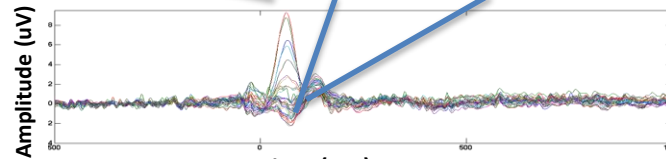
100 ms

135 ms

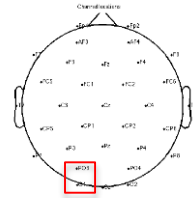
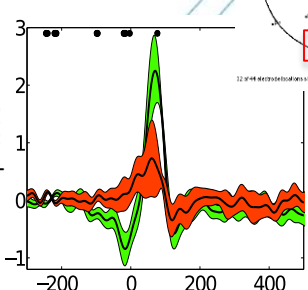


Amplitude (uV)

Time (sec)



Amplitude

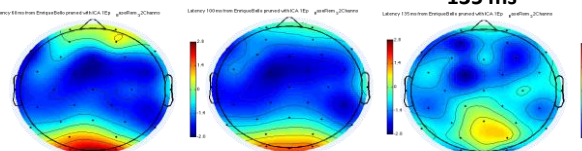


AD patients

60 ms

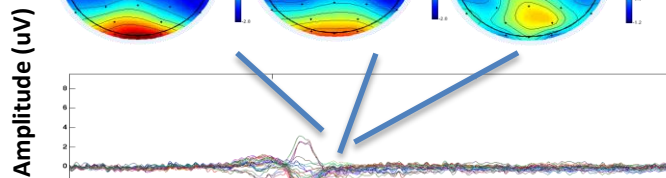
100 ms

135 ms



Amplitude (uV)

Time (sec)

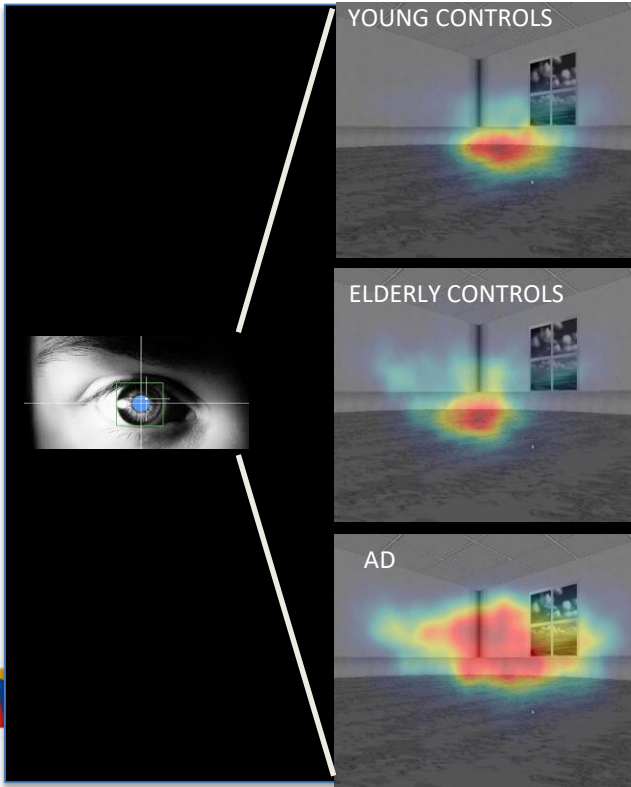


fERP ACTIVITY IN THE OCCIPITAL AREAS IS REMARKABLY ALTERED IN AD PATIENTS

YOUNG CONTROLS

ELDERLY CONTROLS

AD



Dr. Enzo Brunetti

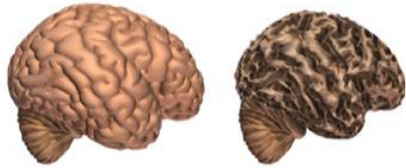


WHAT HAPPENS MOLECULARLY IN THE AD BRAIN?

HISTOPATHOLOGICAL HALLMARKS

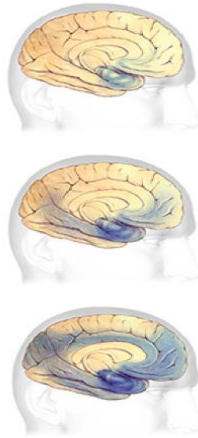
Normal brain

Advanced AD

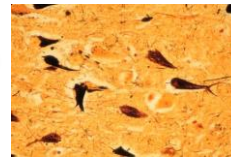


Comparison

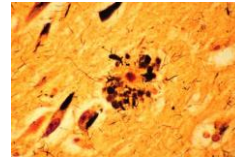
Progression of lesions



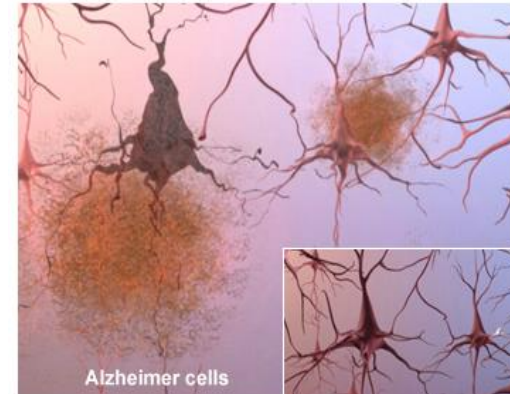
Neurofibrillary tangles



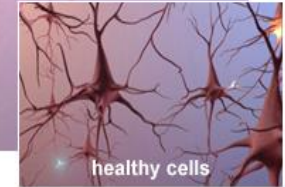
Senile plaques



SYNAPTIC FAILURE



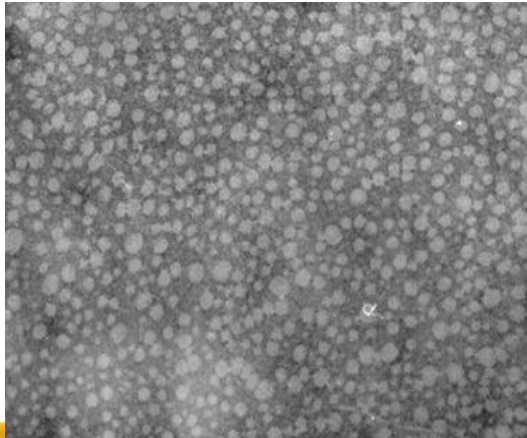
Alzheimer cells



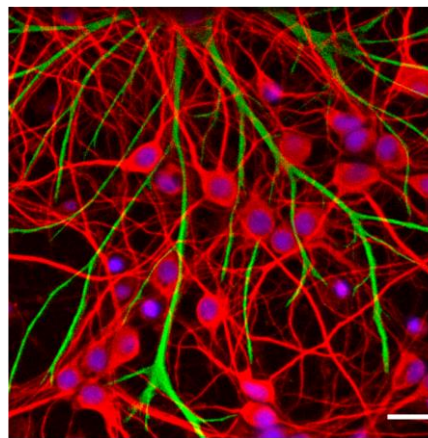
healthy cells

A β Os BIND TO HIPPOCAMPAL NEURONS...

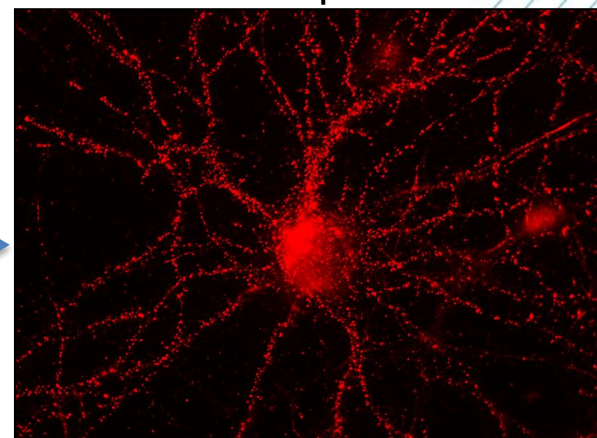
A β Os (AMYLOID AGGREGATES)



Primary hippocampal culture

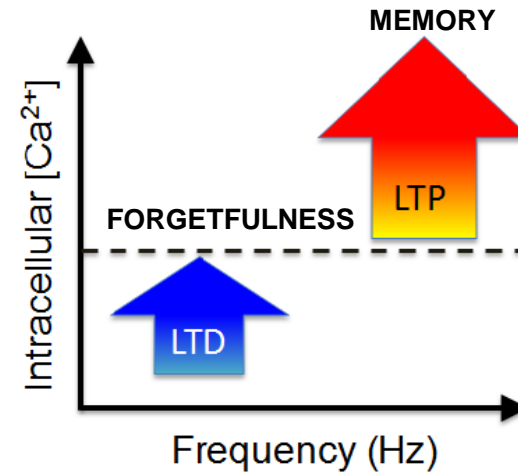
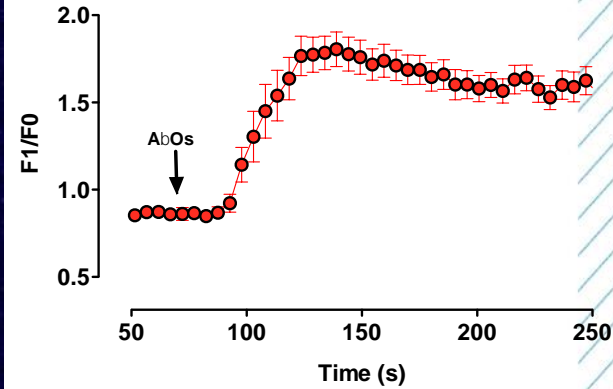
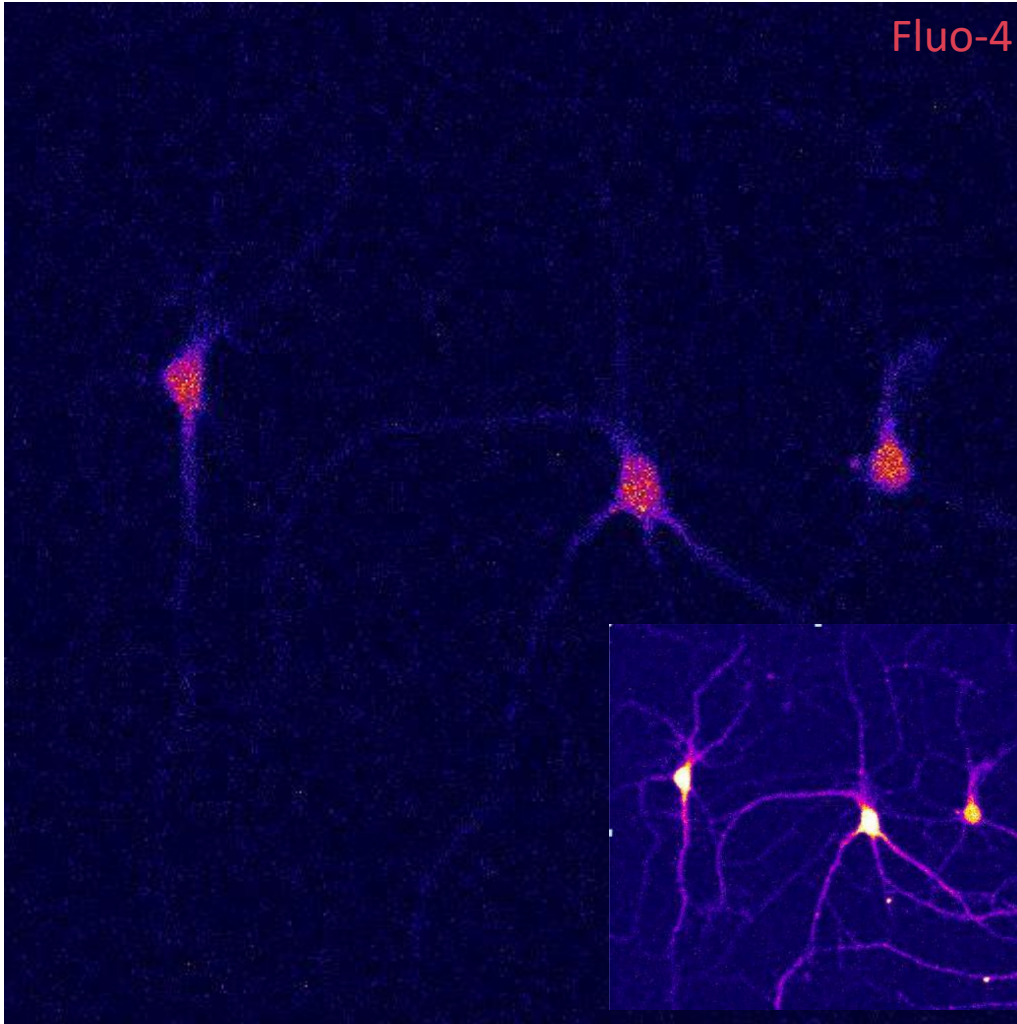


Anti-A β Os



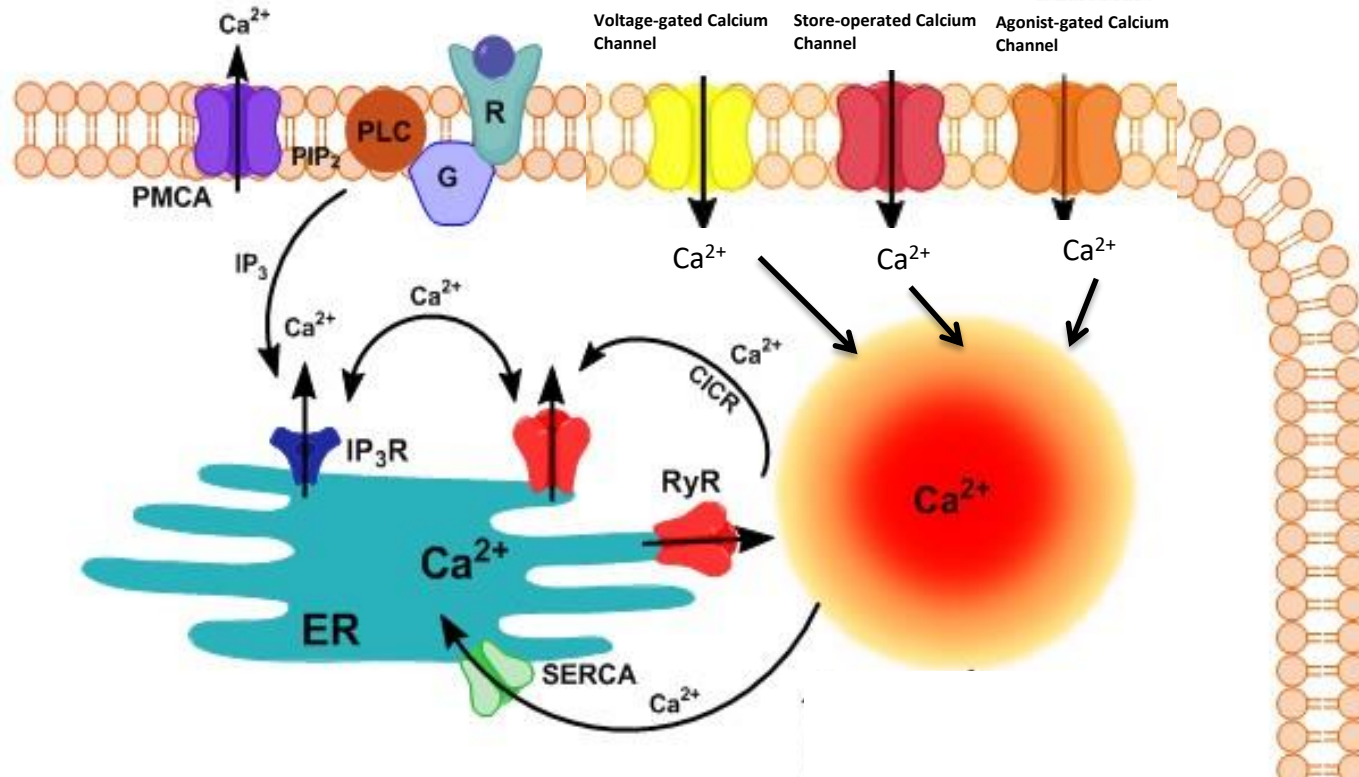
Paula-Lima et al., 2011
Antiox. & Redox Sig.

...AND INDUCE LOW-INTENSITY LONG-LASTING CYTOPLASMIC Ca^{2+} SIGNALS

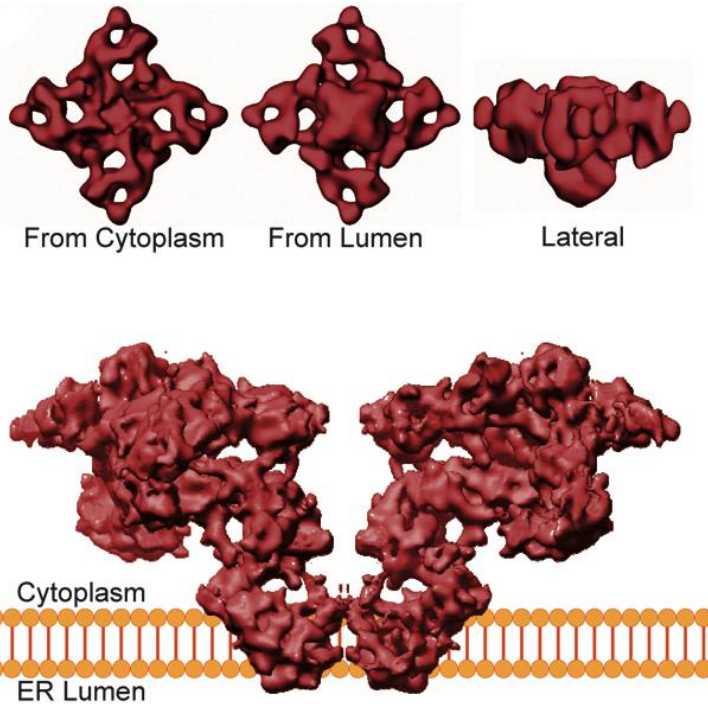


Paula-Lima et al., 2011, Antiox. & Redox Sig.
San Martin et al., 2012, Neurodegenerative diseases
Paula-Lima et al., 2014, Antiox. & Redox. Sig.

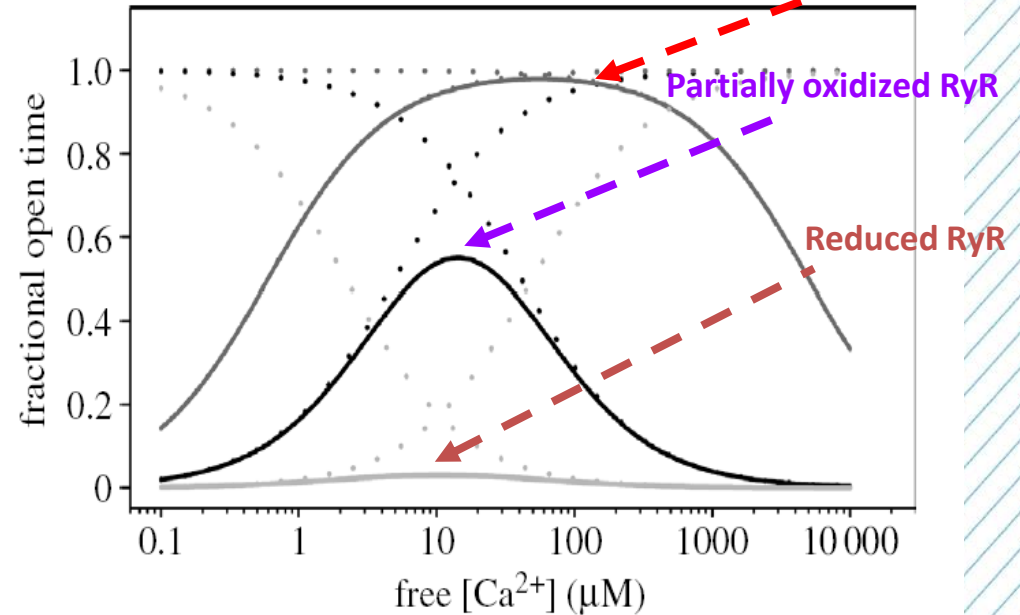
Ca²⁺ SIGNALS GENERATED BY Ca²⁺ RELEASE FROM THE ENDOPLASMIC RETICULUM (RyR, IP3R)



ENDOPLASMIC RETICULUM RESIDENT RYANODINE RECEPTORS (RyR) Ca²⁺ CHANNELS



Paula-Lima, Adasme & Hidalgo 2014
Antiox. & Redox. Sig.



Marengo et al., 1998, *Biophys. J.*

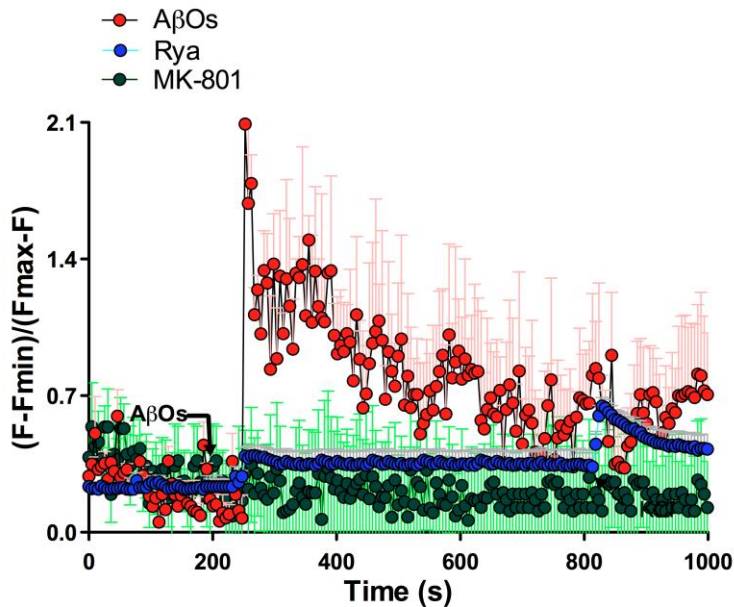
- ❖ Ca²⁺ is the main agonist of RyR
- ❖ Three isoforms (RyR1, RyR2 and RyR3) are present in the brain.
- ❖ RyR is a cellular redox sensor

Ca²⁺ SIGNALS INDUCED BY A β Os REQUIRE FUNCTIONAL NMDAR AND RyR, AND ARE PREVENTED BY ANTIOXIDANTS

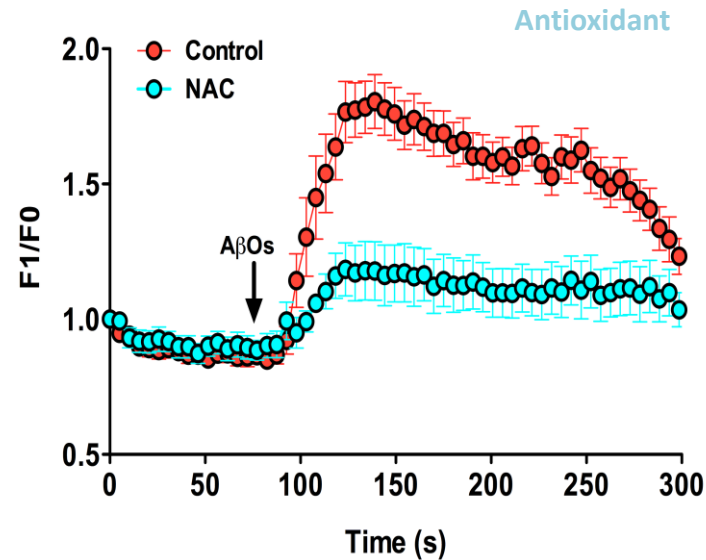


Fluo4

NMDA and RyR-dependent cytoplasmic Ca²⁺ signals

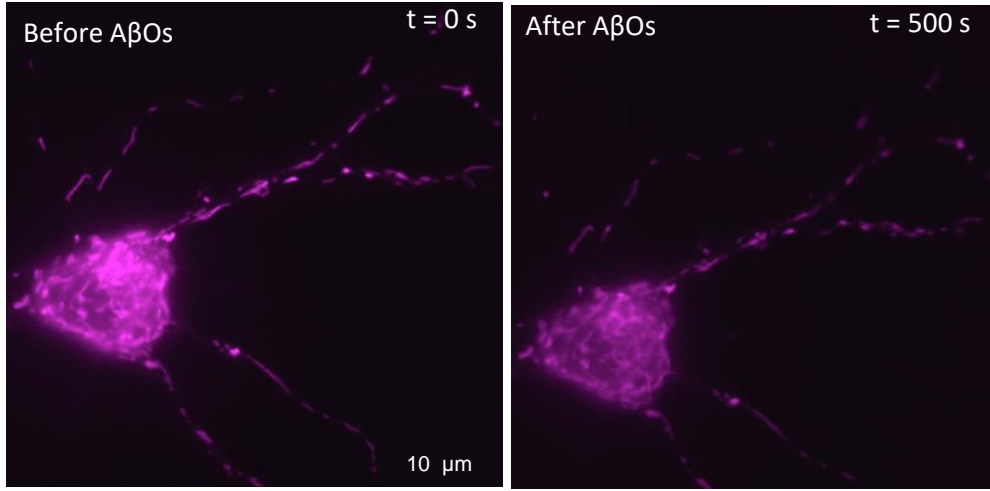


ROS-dependent cytoplasmic Ca²⁺ signals

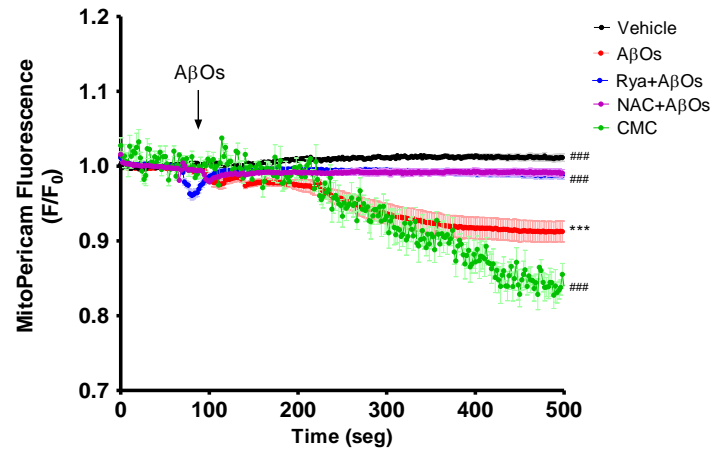
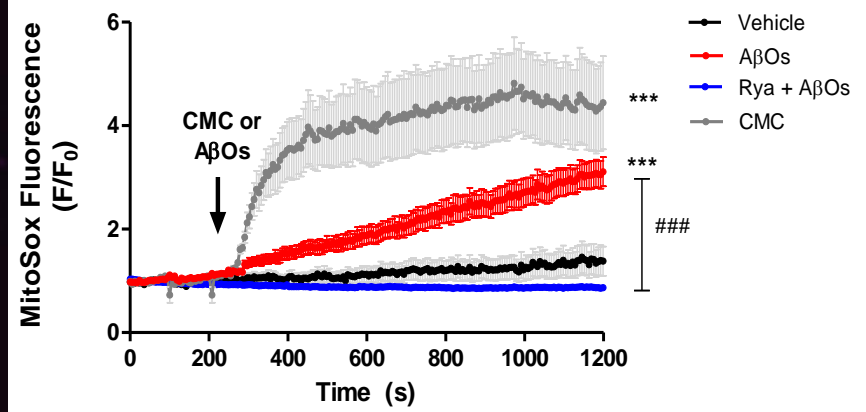


A β O_s PROMOTE RyR-DEPENDENT MITOCHONDRIAL Ca²⁺ LEVELS AND ROS GENERATION, WHICH IS PREVENTED BY RyR INHIBITION AND NAC TREATMENT

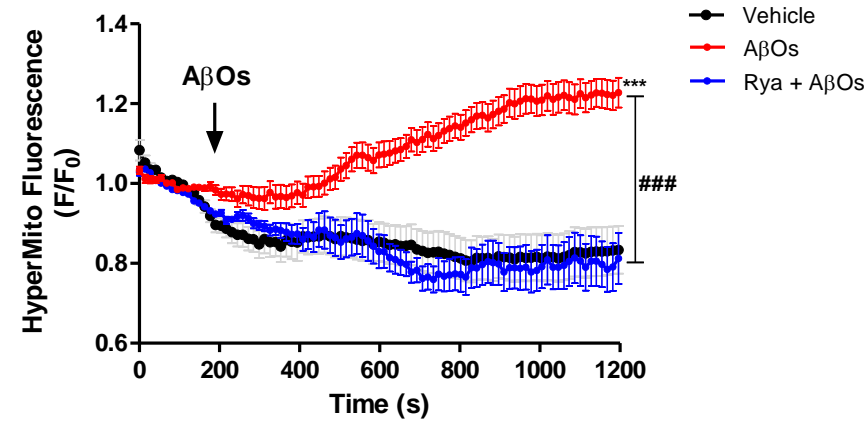
MitoPericam



MitoSOX

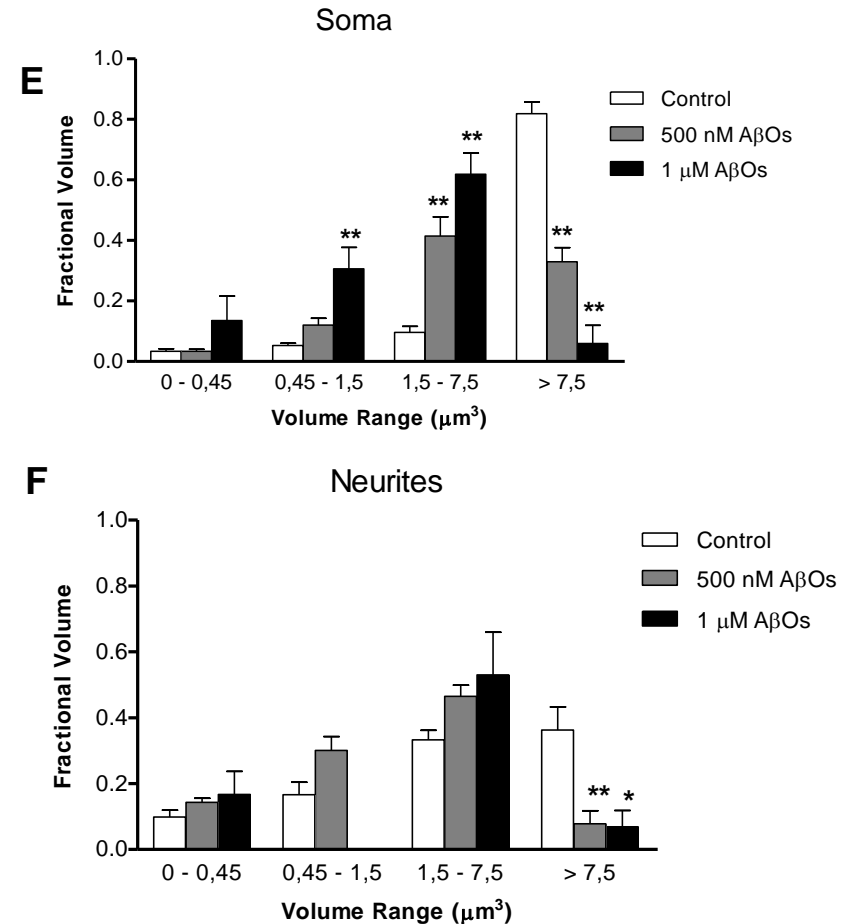
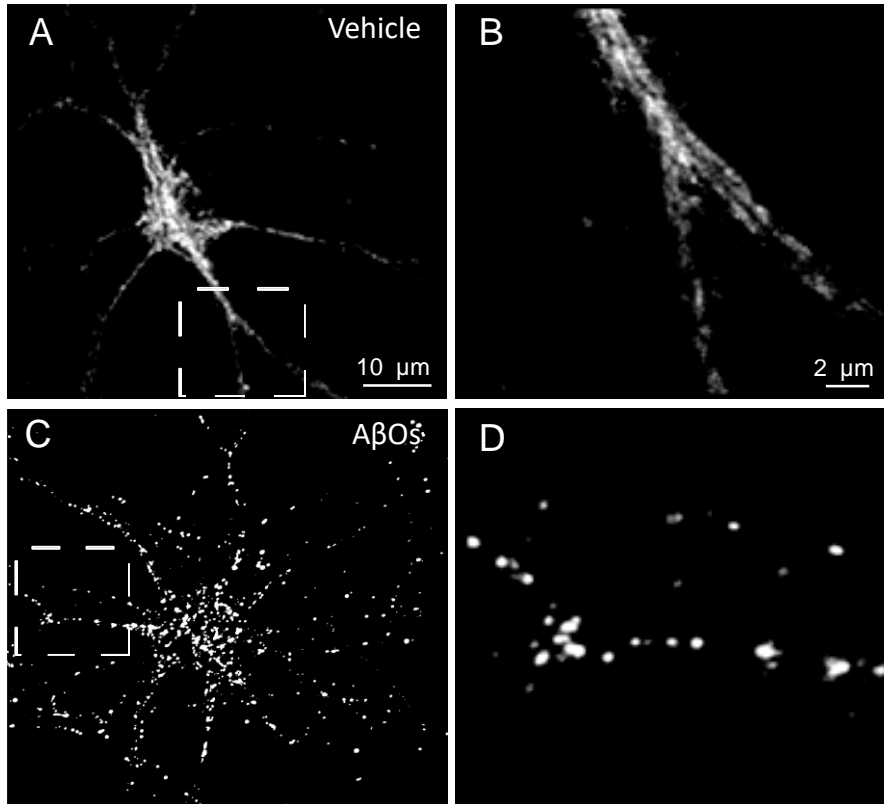


HyperMito

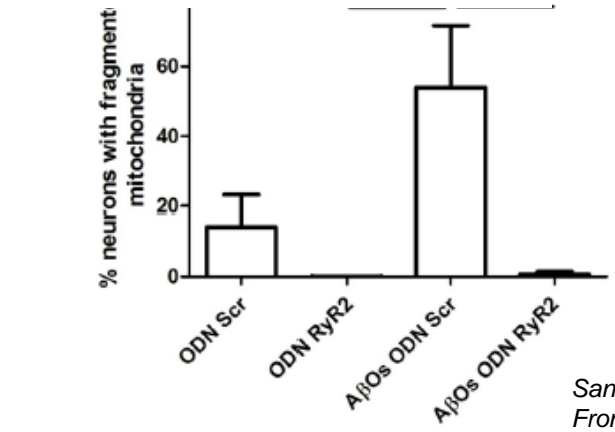
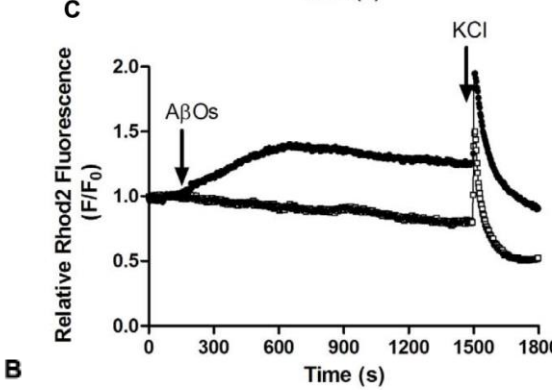
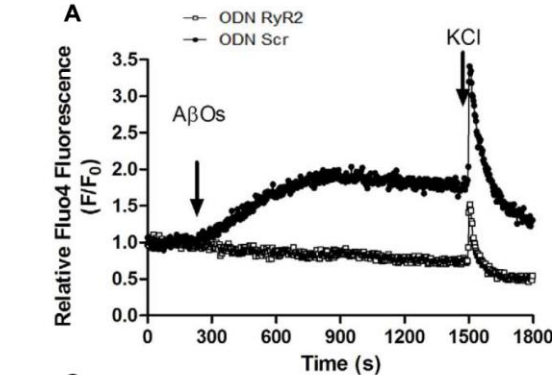
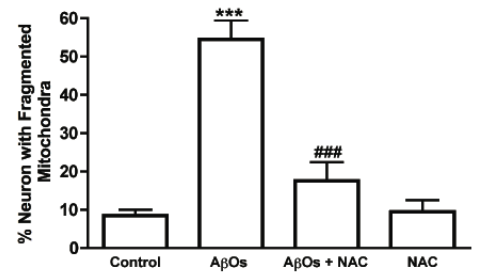
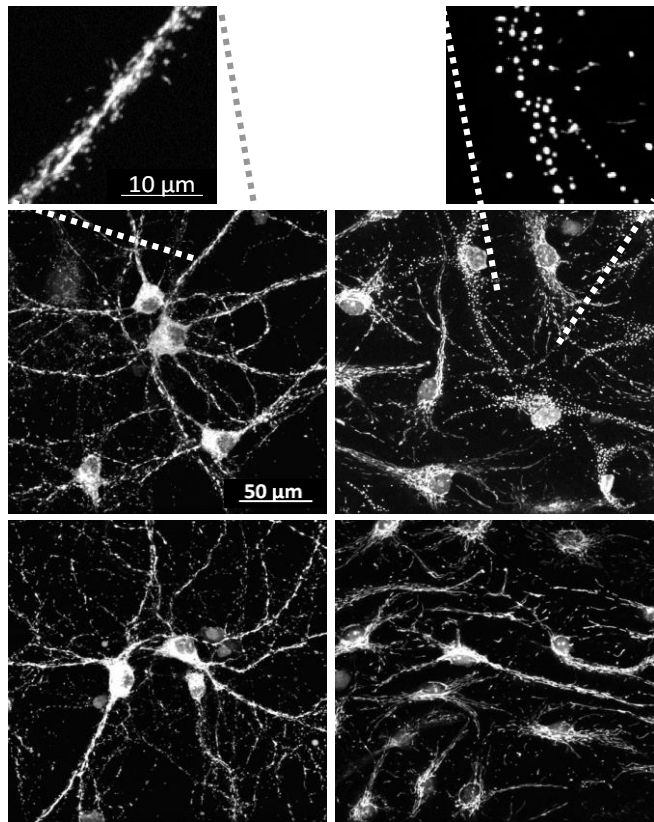


San Martin, Veloso et al., 2017
Frontiers in Molecular Neurosci.

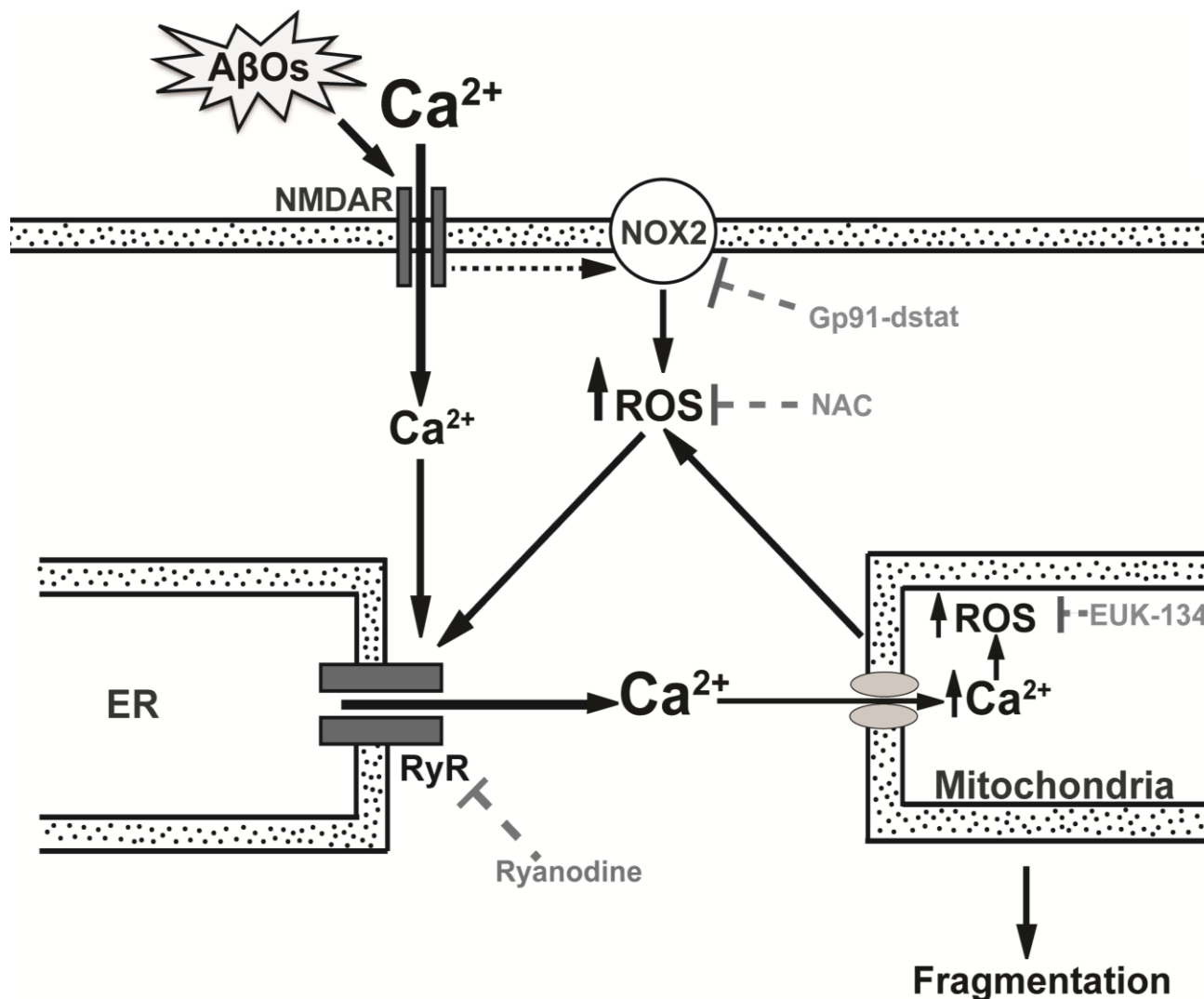
A β O_s INDUCE MITOCHONDRIAL FRAGMENTATION ALTERING NETWORK CLUSTERING IN PRIMARY HIPPOCAMPAL NEURONS



ANTIOXIDANTS AND RyR2 DOWNREGULATION PREVENTS MITOCHONDRIAL FRAGMENTATION INDUCED BY AβOs



CONCLUSIONS



RYR-MEDIATED CA²⁺ RELEASE IS A KEY COMPONENT IN THE MITOCHONDRIAL CA²⁺ AND ROS INCREASES AND FRAGMENTATION INDUCED BY ABOS.

AβOs PRODUCE REDOX-AND CA²⁺-DEPENDENT FUNCTIONAL ALTERATIONS IN THE RAT HIPPOCAMPUS, LEADING TO THE ABNORMAL PATTERNS OF BRAIN ACTIVITY, CAUSING DEFECTIVE SPATIAL MEMORY.



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ACKNOWLEDGMENTS



FONDECYT 1150736; CONICYT 79090021; U-APOYA U-MODERNIZA; FONDECYT 3085025; FONDECYT/FONDAP 15010006; FONDECYT1100052; BNI P-09-015F

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THANK YOU FOR YOUR ATTENTION!



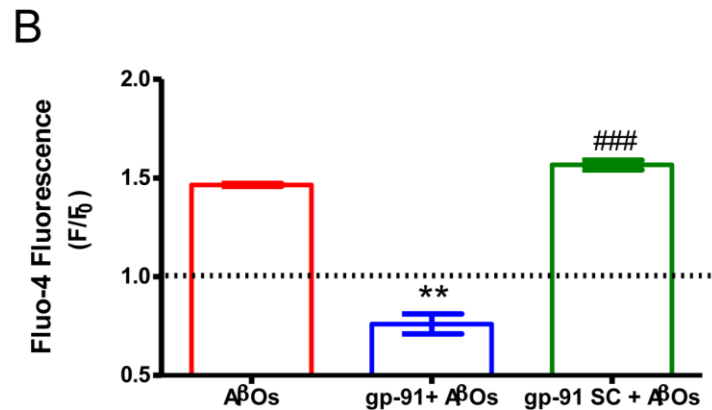
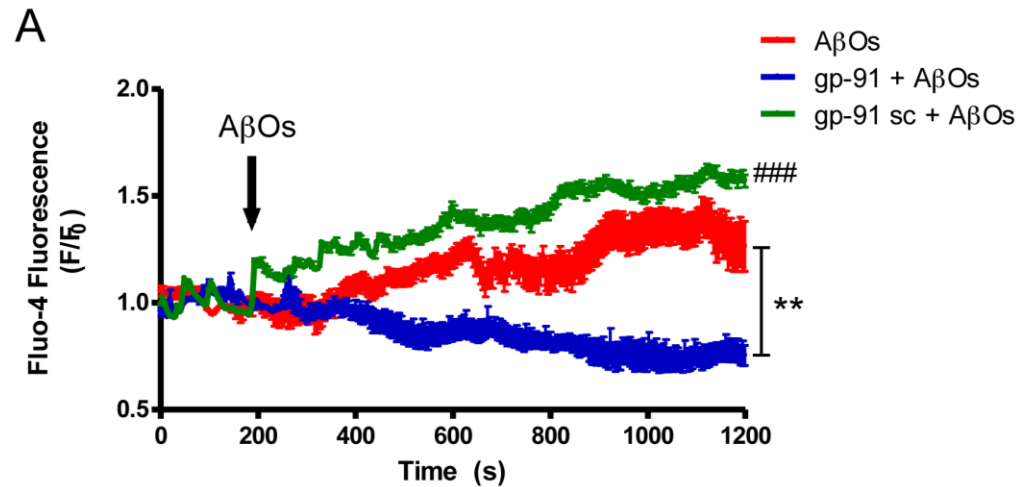
University of Chile



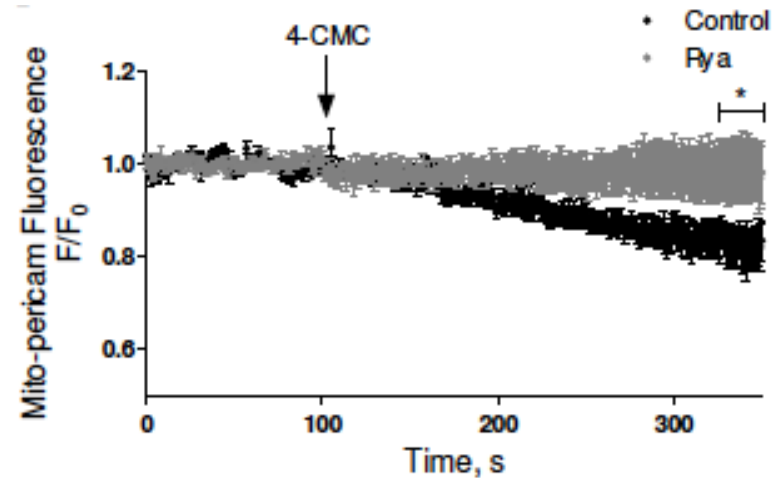
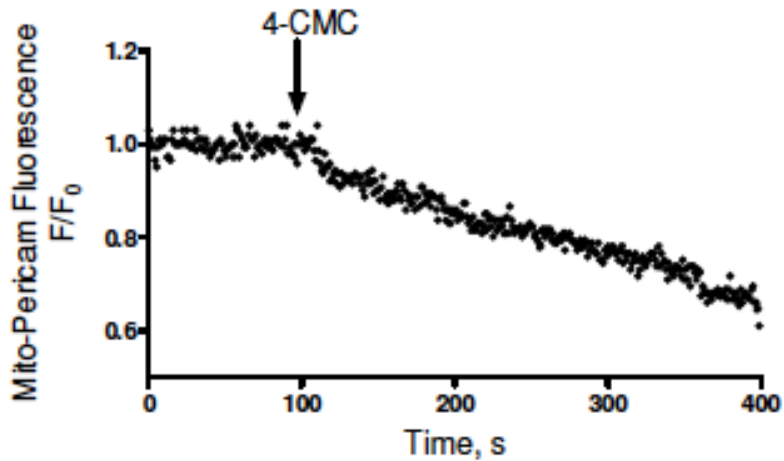
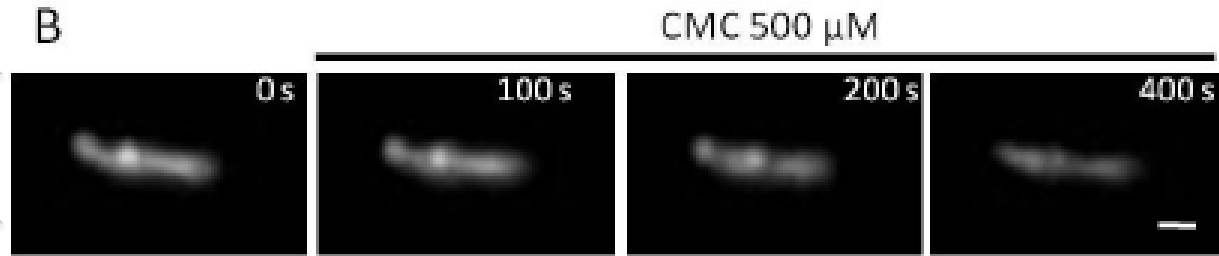
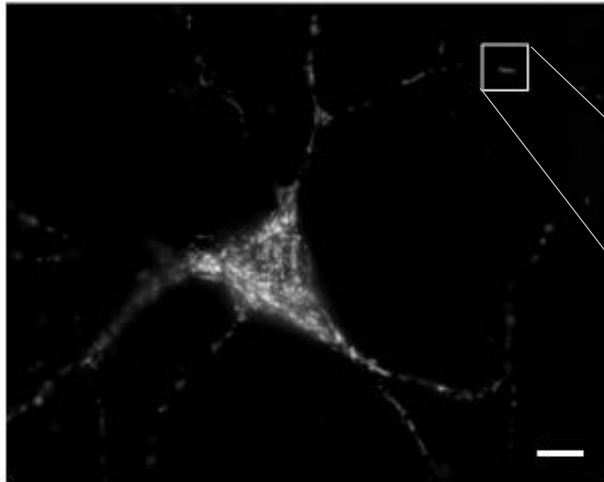
Federal University of Rio de Janeiro



NOX2 INHIBITION PREVENTS THE EMERGENCE OF A β O_s-INDUCED CYTOPLASMIC CA²⁺ SIGNALS.



MITOCHONDRIAL Ca^{2+} LEVELS INCREASE IN RESPONSE TO RyR AGONIST

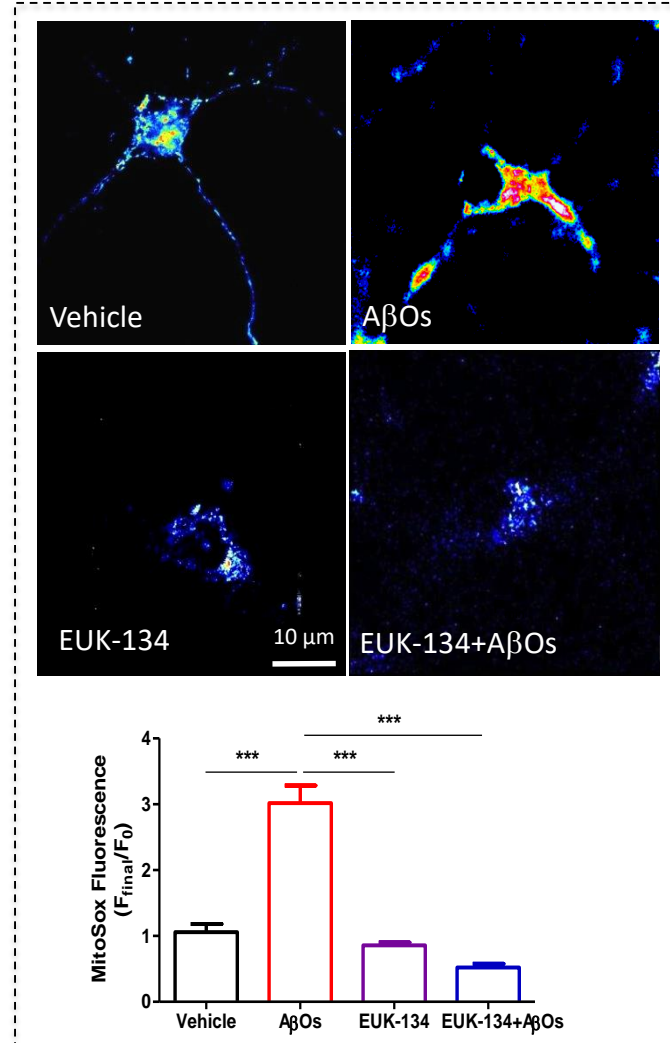
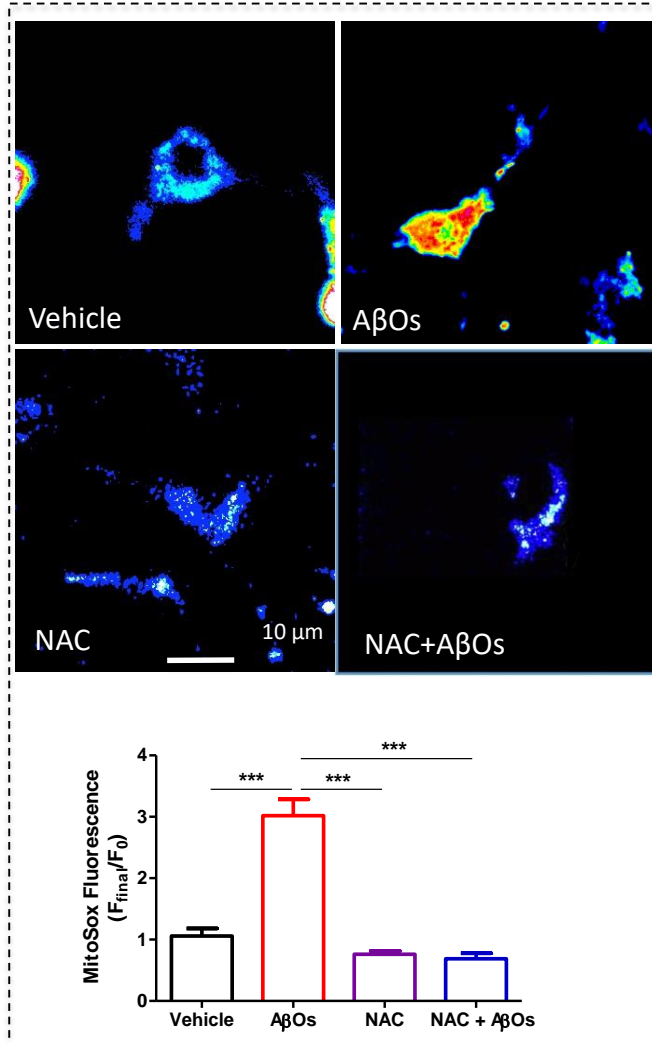


RyR-mediated CICR increases mitochondrial Ca^{2+}



San Martin, Paula-Lima et al, , 2014
*Frontiers in Molecular
Neuroscience*

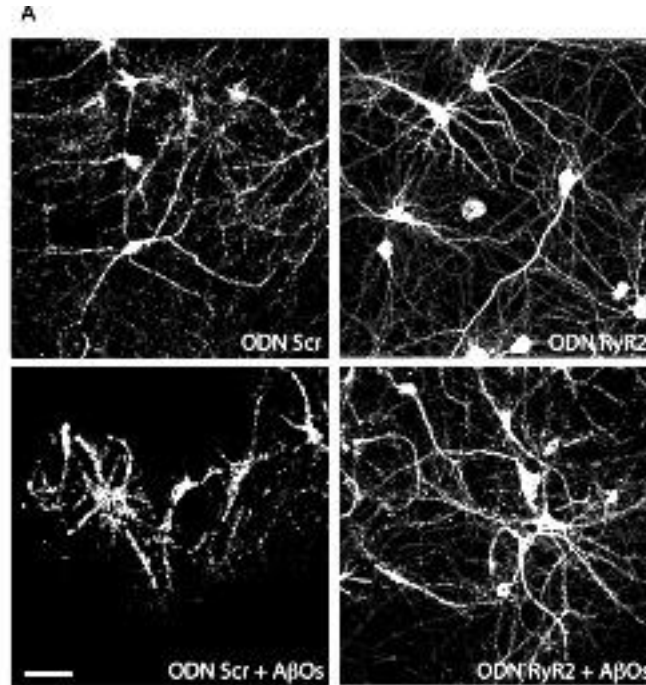
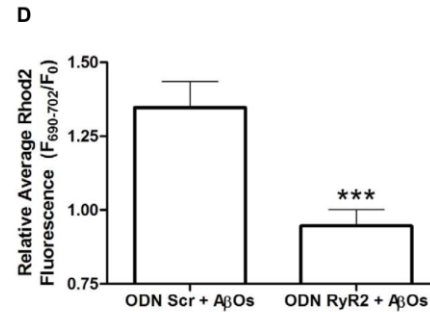
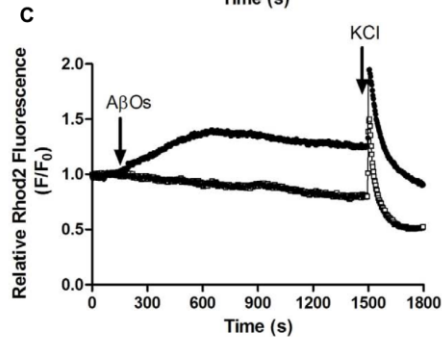
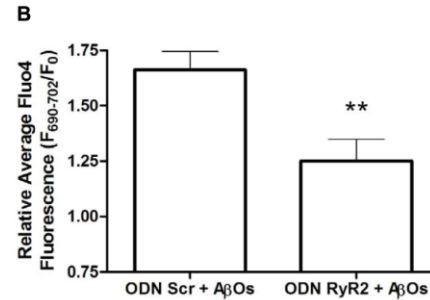
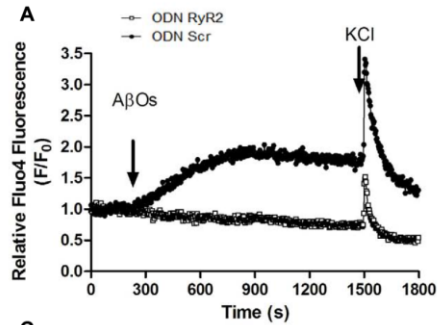
A β Os INDUCE INCREASES IN MITOCHONDRIAL ROS (peroxide and superoxide) LEVELS, WHICH IS PREVENTED BY ANTIOXIDANTS



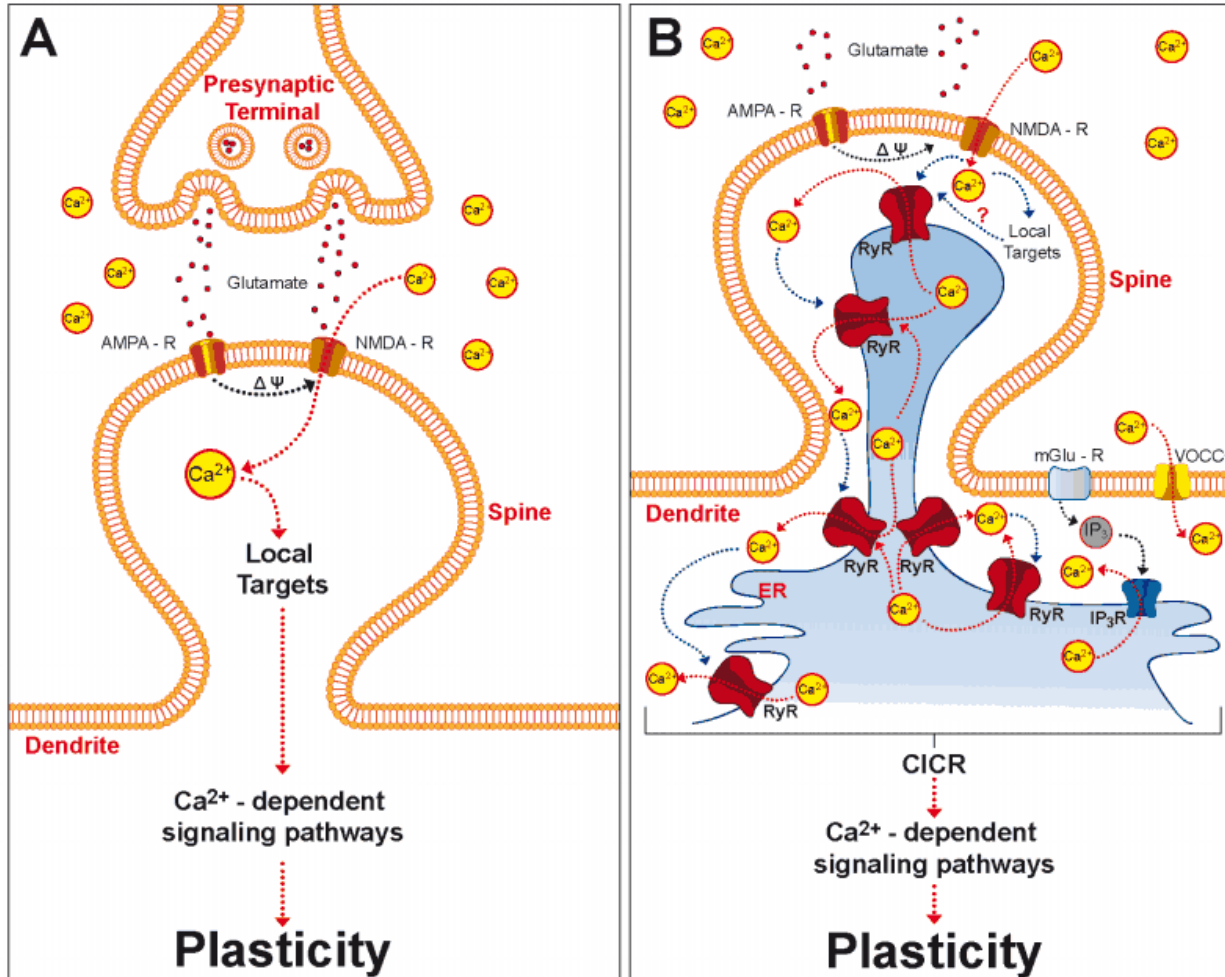
San Martin, Veloso et al., 2017
Frontiers in Molecular Neurosci



RyR2-MEDIATED Ca^{2+} RELEASE PARTAKE IN THE MITOCHONDRIAL DYSFUNCTION CAUSED BY $\text{A}\beta\text{Os}$



HOWEVER, RYR2-MEDIATED CICR IS CRUCIAL TO SYNAPTIC PLASTICITY



Paula-Lima et al., 2014
Antiox. & Redox. Sig.

HOWEVER, RyR ARE REQUIRED TO SPINE REMODELING AND MEMORY FORMATION

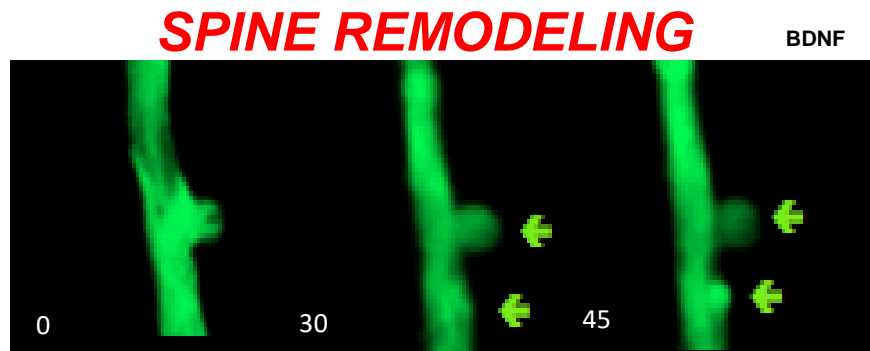


Involvement of ryanodine receptors in neurotrophin-induced hippocampal synaptic plasticity and spatial memory formation

Tatiana Adasme^{a,1}, Paola Haeger^{a,1}, Andrea C. Paula-Lima^a, Italo Espinoza^a, M. Mercedes Casas-Alarcón^a, M. Angélica Carrasco^{a,b,2}, and Cecilia Hidalgo^{a,b,3}

^aCentro de Estudios Moleculares de la Célula, Facultad de Medicina, and ^bPrograma de Fisiología y Biofísica, Instituto de Ciencias Biomédicas, Universidad de Chile, 838-0453 Santiago, Chile

- *BDNF-induced spine remodeling require functional RyR.*



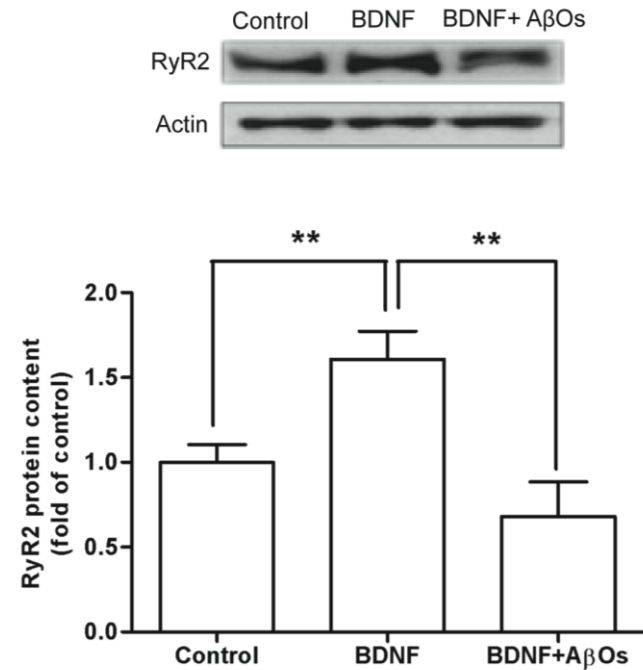
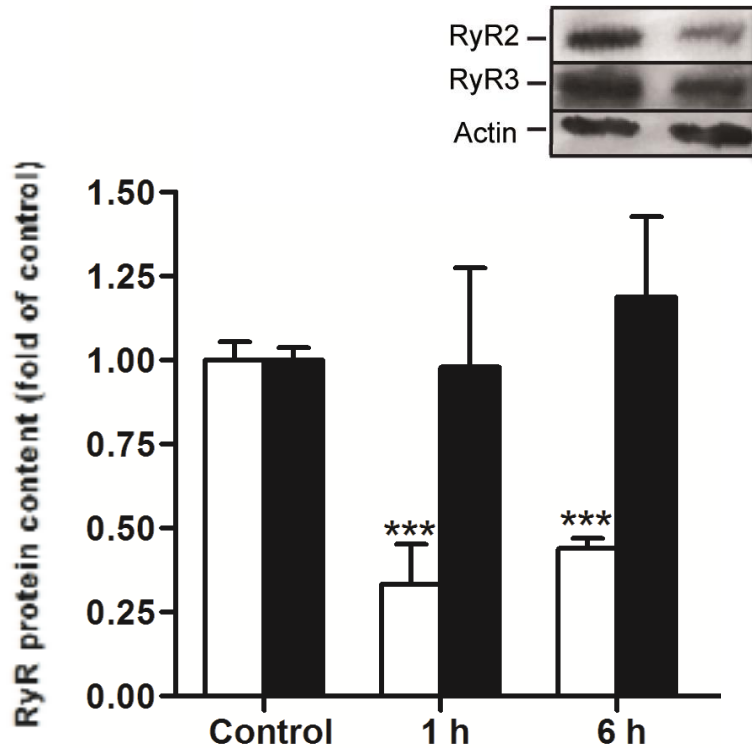
DOES ALTERED
A β O_s-INDUCED
Ca²⁺ RELEASE
IMPAIR SPINE
GROWTH AND RyR
EXPRESSION?

- *BDNF treatment and memory training enhance RyR2 and RyR3*





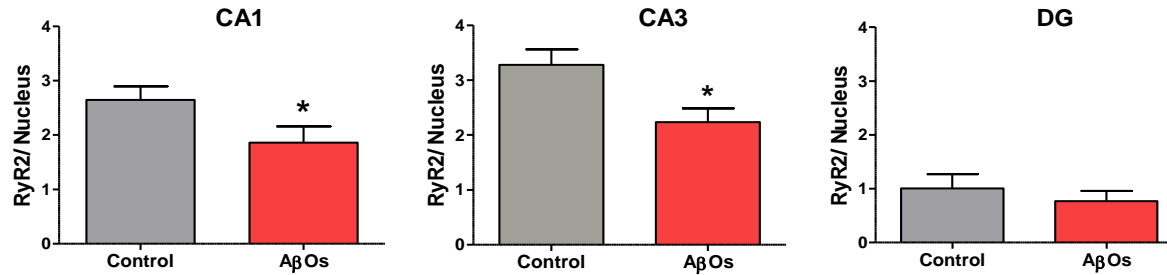
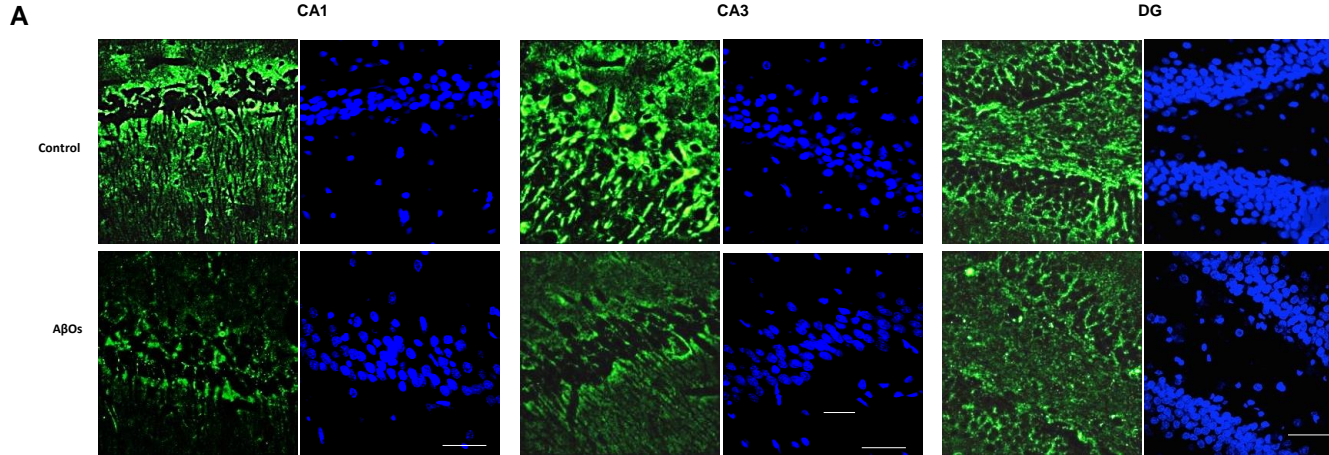
A β Os DOWN-REGULATE NEURONAL RyR2 AND PREVENT THE INCREASES IN RyR2 EXPRESSION INDUCED BY BDNF



A β Os DECREASE HIPPOCAMPAL RYR2 PROTEIN CONTENT IN VIVO IMPAIRING SPATIAL MEMORY

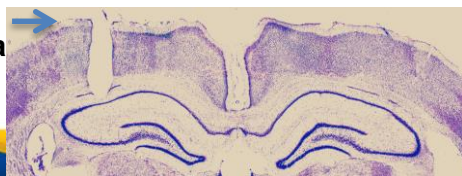


RyR2 EXPRESSION (Immunofluorescence)

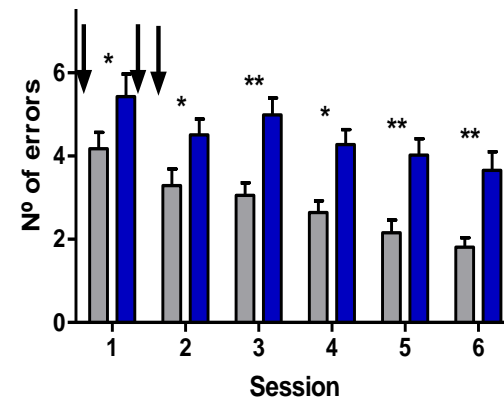
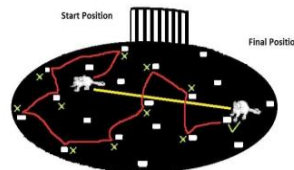


BILATERAL INTRAHIPPOCAMPAL INJECTION (CRESYL VIOLET)

Guide
cannula



OASIS MAZE

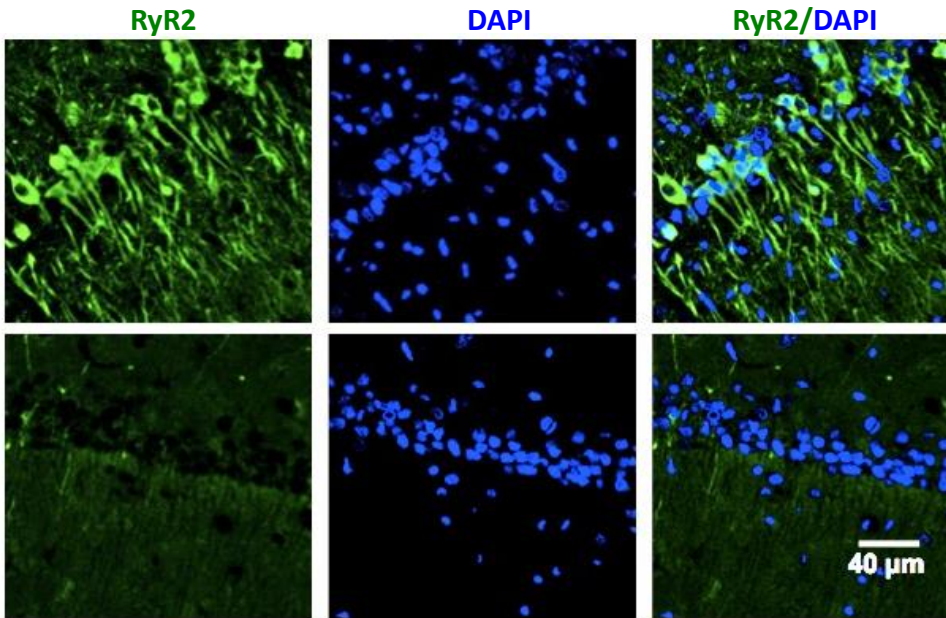


Jamileth More
(PhD student)

SELECTIVE KNOCKDOWN OF RyR2 IMPAIRS SPATIAL MEMORY PROCESSES



Scrambled
antisense
oligonucleotides



Anti-RyR2

