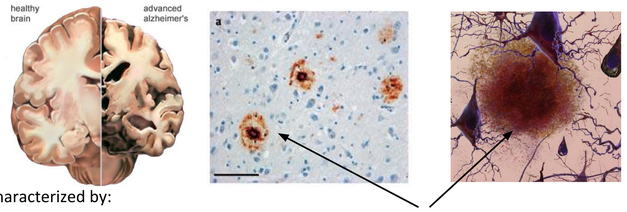
The Effects of Amyloid Beta on AMPA Receptors

Mia Franco, Dr. Kim Doré, Dr. Robert Malinow
University of California San Diego, San Diego, CA, 9203

Introduction

The AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) subtype excitatory amino acid receptor is responsible for mediating fast synaptic transmission in most CNS (central nervous system) excitatory synapses. When dysfunction occurs in this receptor it can result in mental diseases such as, Alzheimer's. Thus, the purpose for this study was to observe how amyloid beta (Aβ, a protein that is the main component of amyloid plaques found in the brains of Alzheimer's patients) affects postsynaptic receptors particularly the AMPA receptor. In order to do this, we introduced the Aβ in neurons with a virus. We used neurons from two kinds or hippocampal preparations. Through the use of fluorescence lifetime imaging (FLIM) we we're able to take images of neurons expressing the GluR2 subunit of the AMPA receptor tagged with a GFP (green fluorescent protein) variant that is pH sensitive. More specifically, we we're able to measure the effects of Aβ on the fluorescence lifetime of this GFP (SEP) in spines and dendrites.

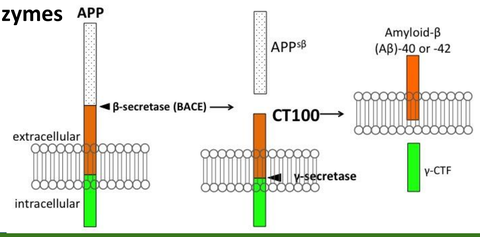
Alzheimer's disease



Characterized by:

- Significant neurodegeneration
- Amyloid plaques (comprising amyloid-β)

Aβ comes from the cleavage of the Amyloid Precursor Protein (APP) by 2 enzymes

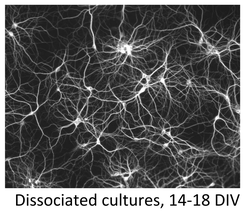


It is necessary for β secretease and γ secretease to cut off from APP because without it, amyloid beta wouldn't be able to form.

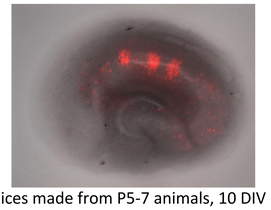
Methods

Hippocampal preparations used: dissociated cultures and organotypic slices

The hippocampus is important for memory formation and is affected early on in Alzheimer's patients

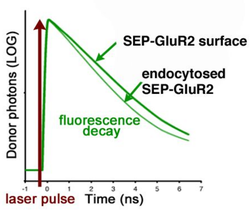


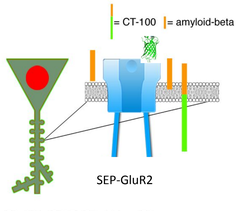
Dissociated cultures, 14-18 DIV
Made from P0 animals (rats)
Lipofection (lipid+DNA) of SEP-GluR2



Slices made from P5-7 animals, 10 DIV
Injection a virus expressing SEP-GluR2 (and CT84/CT100) with a glass needle

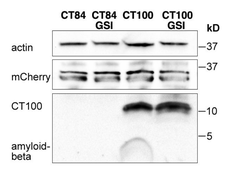
Overexpression of SEP-GluR2 to detect endocytosis with fluorescence lifetime imaging (FLIM)

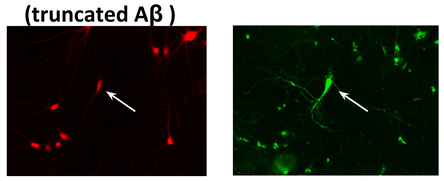




GluN1-GFP/GluN1-mCherry
Sindbis CT100,NLS-mCherry inf.

Infection of neurons with a virus expressing CT100 or CT84 (truncated Aβ)

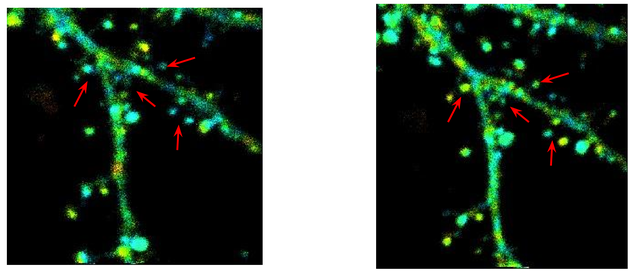




Here are some images showing infection in red and a transfected neuron in green. By taking a closer look we can measure the fluorescence lifetime in single spines and see how amyloid beta can affect AMPA receptors. To the right, you'll see a western blot showing infection as well.

Results

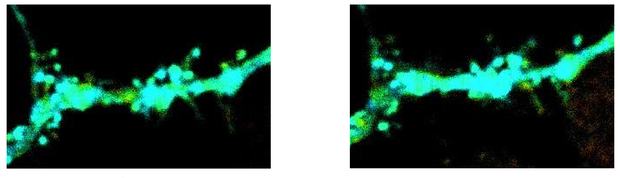
Insulin stimulation induces GluR2 endocytosis



Before

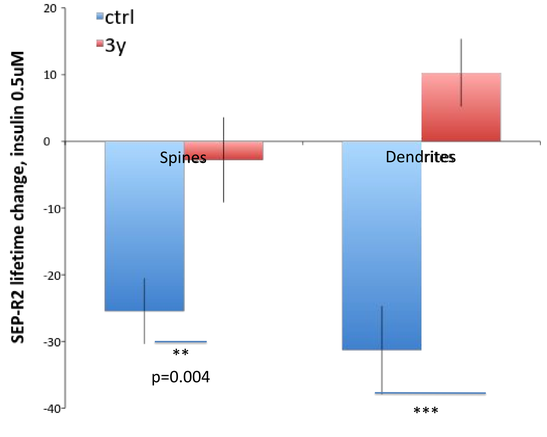
after 20 min 0.5uM insulin

Incubation with a peptide that blocks GluR2 phosphorylation blocks the effect

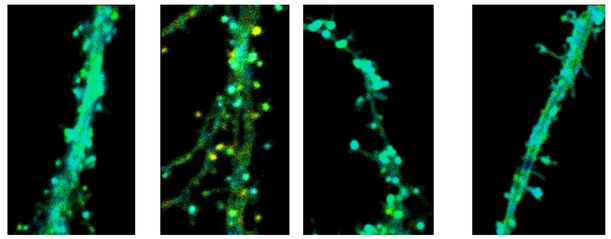


Before
Neurons incubated with 0.5uM 3Y peptide

After 20 min 0.5uM insulin

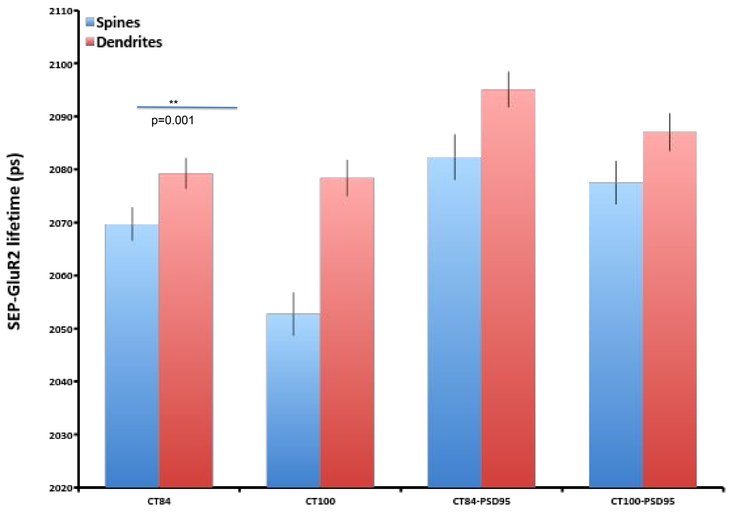


PSD-95 blocks GluR2 endocytosis produced by Aβ overexpression



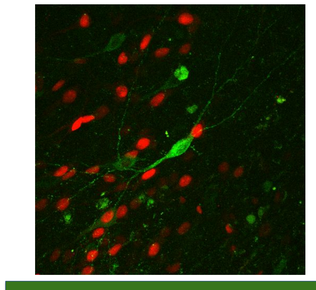
CT84 CT100 CT84-PSD-95 CT100-PSD-95

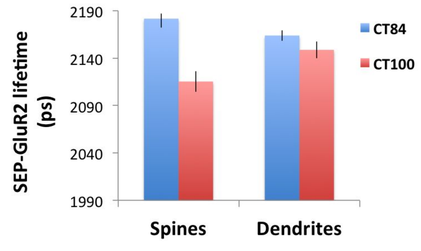
Here, we added PSD-95 to the CT100 and the CT84 viruses. PSD-95 is a protein that makes synapses stronger.
PSD-95 blocks the effect of GluR2 endocytosis produced by Aβ overexpression.



Here you can see that the CT100 only affected the spines (blue) in a significant way, decreasing the lifetime of the SEP-GluR2. However, once we added PSD-95 there was a significant increase in the lifetime. PSD-95 thus protects spines and dendrites from Aβ.

Aβ produces endocytosis in slices





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Acknowledgements

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