In vivo study of abnormal neuronal Ca^{2+} activity in α-synuclein transgenic mice model of Parkinson's disease using 2-photon microscopy

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Summary:

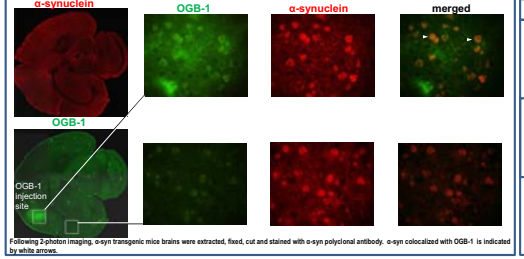
- Parkinson's disease (PD) is a major neurodegenerative disorder characterized by accumulation of toxic α-synuclein (α-syn) oligomers and protofibrils
- α-syn is an abundant presynaptic protein built of 140aa and found aggregated in Lewy Bodies (LBs), the hallmark of PD pathology
- The mechanism through which α-syn may provoke neuronal vulnerability was shown to involve formation of channel-like oligomers that dysregulate the intracellular Ca^{2+} in vitro.

The main objective:

to investigate *in vivo* the role of calcium in mechanisms of neurodegeneration in PD.

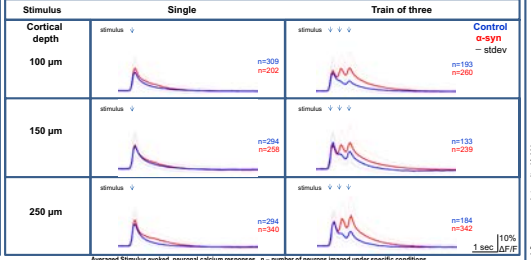
We use 2-photon imaging of α-syn transgenic mice neurons, located in neocortex, where α-syn positive inclusion bodies were seen in PD and in other disorders with LBs.

α-syn co-localized with Ca^{2+} marker in the brains of α-syn tg mice



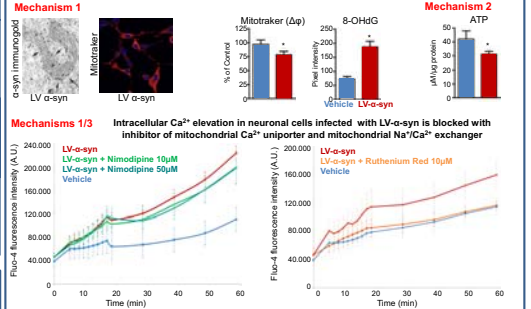
Following 2-photon imaging, α-syn transgenic mice brains were extracted, fixed, cut and stained with α-syn polyclonal antibody. α-syn colocalized with OGB-1 is indicated by white arrows.

Stimulus evoked neuronal Ca^{2+} responses in α-syn mice differ from those of controls



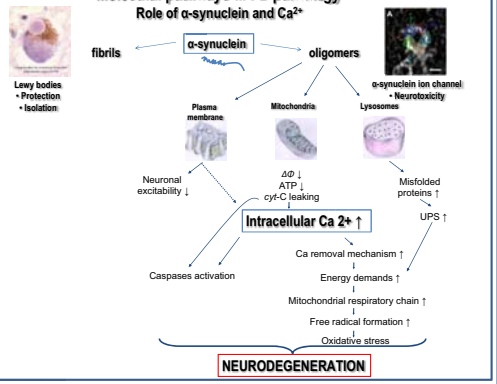
Averaged Stimulus evoked neuronal calcium responses , n – number of neurons imaged under specific conditions

Studies in neuronal culture support general mitochondrial disfunction and high basic Ca^{2+}

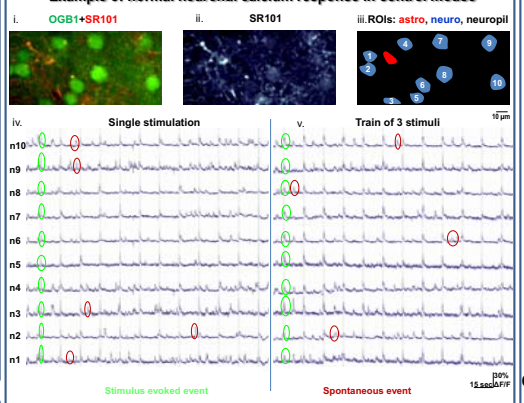


Rat neuroblastoma B103 cells line were infected with LV expressing human wt α-syn . Cellular ATP levels were measured using the ATP luminescent assay kit, Cell Titer-Glo. Mitochondrial morphology and damage to mitochondrial membrane integrity were examined using the membrane permeable dye Mitotracker Red MCXRos. 8-Hydroxy-2'-deoxyguanosine (8-OHdG) was used as a biomarker of oxidative stress. Intracellular calcium was assessed using a Fluo-4 AM dye.

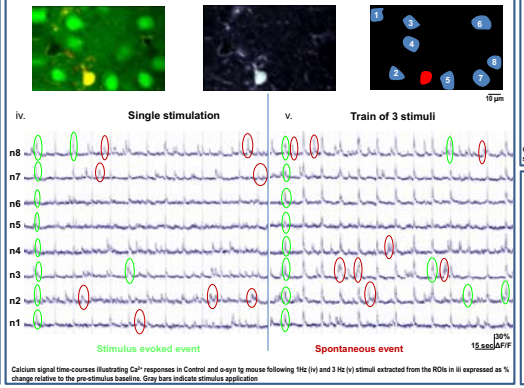
Molecular pathways in PD pathology



Example of normal neuronal calcium response in control mouse

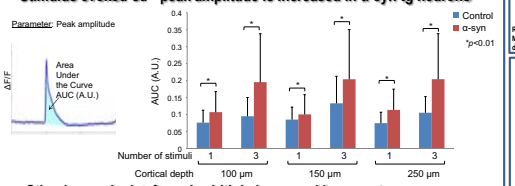


Example of abnormal neuronal calcium response in α-syn mouse

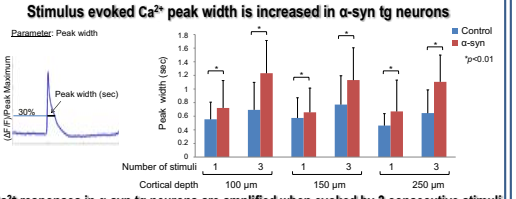


Calcium signal time-courses illustrating Ca^{2+} responses in Control and α-syn tg mouse following 1Hz (iv) and 3 Hz (v) stimuli extracted from the ROIs in iii expressed as % change relative to the pre-stimulus baseline. Gray bars indicate stimulus application

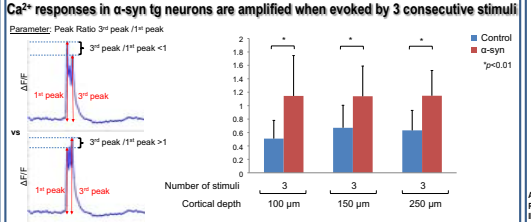
Stimulus evoked Ca^{2+} peak amplitude is increased in α-syn tg neurons



Stimulus evoked Ca^{2+} peak width is increased in α-syn tg neurons



Ca^{2+} responses in α-syn tg neurons are amplified when evoked by 3 consecutive stimuli

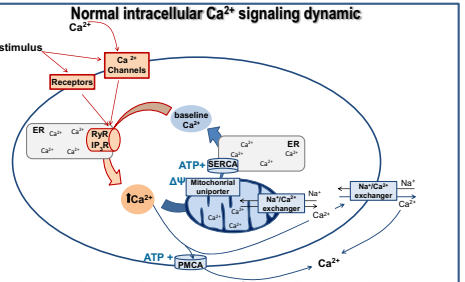


Graphic representation of parameters characterizing stimulus evoked responses of α-syn tg neurons vs control mice neurons. Data analysis was performed with Matlab software . Error bars represent standard deviation.

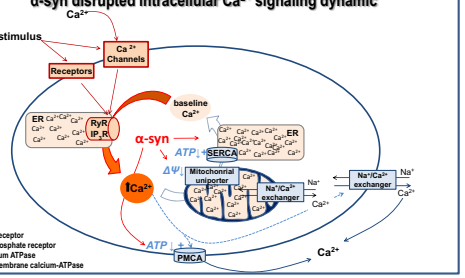
Multiple mechanisms may lead to α-syn tg neuronal calcium abnormalities

1. α-syn accumulates in mitochondria → ⇩ functional mitochondria → ⇩ Ca^{2+} buffering capacity in the neurons → ⇧ Ca^{2+} entry and ⇩ Ca^{2+} clearance → ⇧ Ca^{2+} peak amplitude, peak width and Ca^{2+} response amplification following multiple stimuli
2. ⇩ ATP → ⇩ Ca^{2+} clearance by ATPases → ⇧ Ca^{2+} peak width and Ca^{2+} response amplification following multiple stimuli
3. ⇧ Basic Ca^{2+} level in Intracellular stores → ⇧ ATP requirements → ⇩ Ca^{2+} clearance → ⇧ Ca^{2+} peak width and Ca^{2+} response amplification following multiple stimuli

Normal intracellular Ca^{2+} signaling dynamic



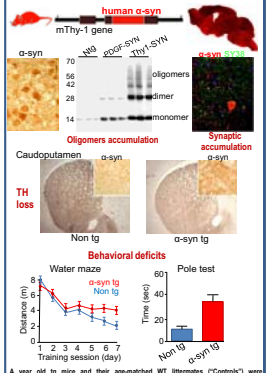
α-syn disrupted intracellular Ca^{2+} signaling dynamic



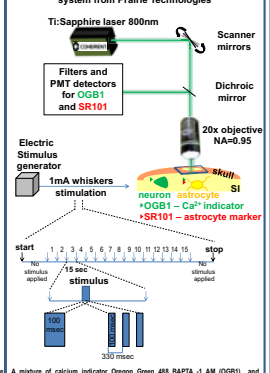
Abbreviations:
RyR- ryanodine receptor
IP3R- 1,4,5-trisphosphate receptor
SERCA- ER calcium ATPase
PMCA- plasma membrane calcium-ATPase

Hypothesis: α-syn oligomers dysregulates neuronal Ca^{2+} homeostasis *in vivo*

Experimental approach



A year old tg mice and their age-matched WT littermates ("Controls") were anesthetized with α-chloralose. HR, BP, expired CO_2 and t were continuously monitored. Respiration was aimed to maintain pCO_2 between 35 and 45 mmHg.



A mixture of calcium indicator Oregon Green 488 BAPTA -1 AM (OGB1) and astrocytic marker SR101 were microinjected in the cortical layer 2/3 calcium indicator Oregon Green 488 BAPTA -1 AM; SI- Primary Somatosensory Cortex

Conclusions:

- Tg mice neurons, expressing human α-syn, exhibit amplification of Ca^{2+} evoked and spontaneous activity as described by significant increase in amplitude and duration of Ca^{2+} transients.
- Ca^{2+} response is mostly amplified in tg neurons excited with the train of 3 Hz stimuli, with each new stimulus added.
- In vitro studies support the involvement of multiple mechanisms in α-syn induced Ca^{2+} homeostasis impairment. Low levels of ATP, impaired mitochondrial membrane potential and increased marker for oxidative stress were detected in LV-α-syn infected neuronal cells. The increase in Ca^{2+} in LV-α-syn infected neuronal cells may be blocked using inhibitor of mitochondrial Ca^{2+} uniporter and Na^+/Ca^{2+} mitochondrial exchanger.
- Further studies will be required to verify whether ER and mitochondria in α-syn infected neurons are overloaded with Ca^{2+}.
- The major consequence of this study is that amplification of Ca^{2+} response amplitude and duration in α-syn tg mice may be used as functional *in vivo* imaging biomarker characterizing α-syn induced PD-related pathology and enabling the objective *in vivo* screening of newly designed neuroregenerative drugs.

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