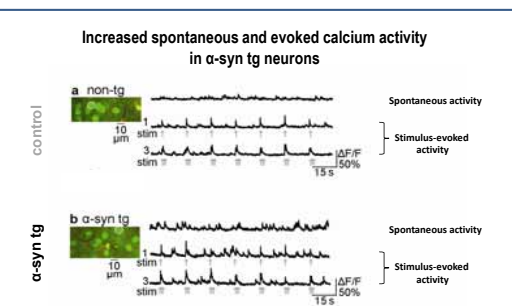


# Novel organic compound targeting misfolded $\alpha$ -synuclein normalized calcium abnormalities in $\alpha$ -synuclein transgenic Parkinson's Disease-like mouse model

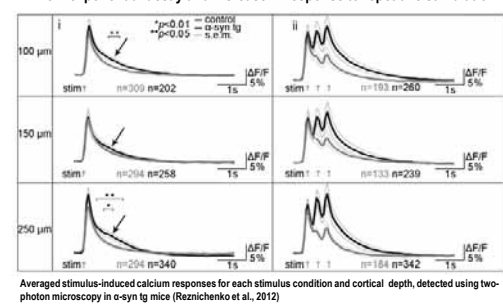
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## Background:

Aberrant aggregation of the presynaptic protein alpha synuclein ( $\alpha$ -syn) has been proposed to underlie the pathogenesis of a host of neurodegenerative disorders including Parkinson's disease, dementia with Lewy bodies and multiple system atrophy. Using *in vivo* two-photon laser scanning microscopy, we have demonstrated that neurons in the barrel cortex of mice expressing WT human  $\alpha$ -syn (Thy-1, line 61 (tg); Masliah et al., 2000; Rockenstein et al., 2002) exhibit pathological calcium activity as a consequence of  $\alpha$ -syn interference with cellular calcium buffering mechanisms.



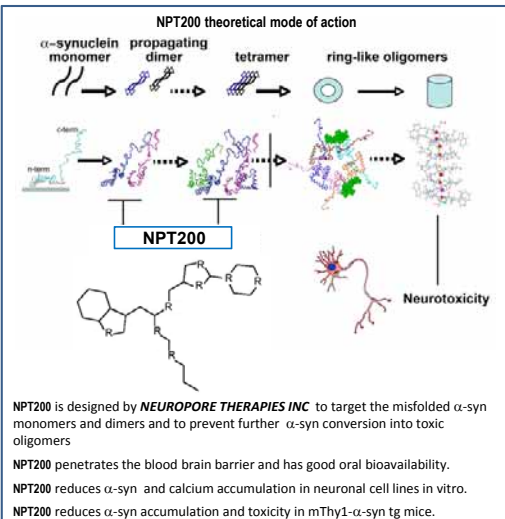
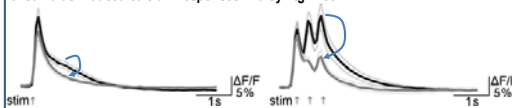
## Evoked calcium activity in $\alpha$ -syn tg mice is characterized by deviation from exponential decay and increase in response to repetitive stimulation



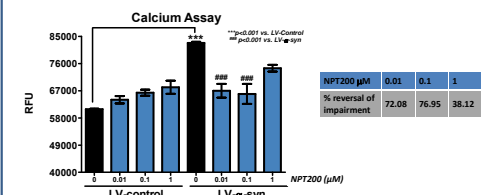
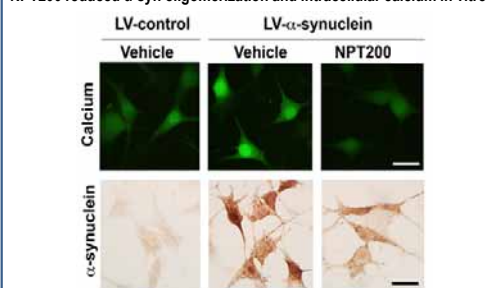
## The main objective:

To use the augmented calcium response in mice expressing hWT  $\alpha$ -syn as an *in vivo* functional biomarker of  $\alpha$ -syn related pathology to explore the therapeutic potential of a novel  $\alpha$ -synuclein stabilizing agent NPT200. To determine whether treatment with NPT200 would normalize the aberrant calcium response in barrel cortex of  $\alpha$ -syn tg mice (Thy-1, line 61).

## Potential therapeutic compound is expected to normalize $\alpha$ -syn related pathology of stimulus-induced calcium responses in $\alpha$ -syn tg mice

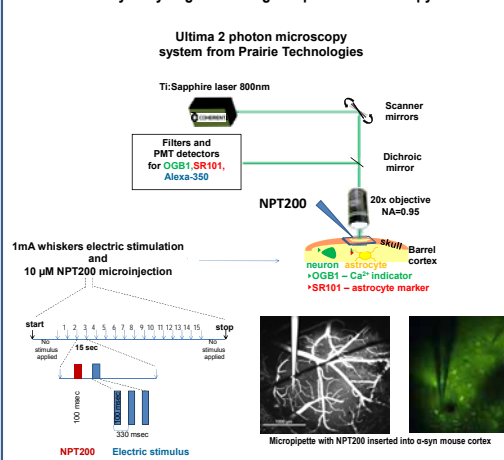


## NPT200 reduced $\alpha$ -syn oligomerization and intracellular calcium in vitro



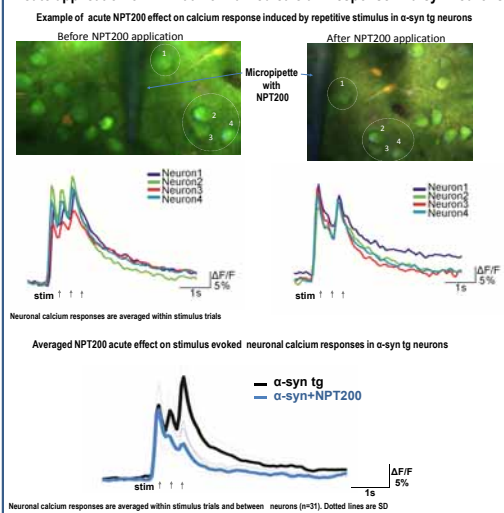
Rat neuroblastoma B103 cell line was infected with LV expressing human wt  $\alpha$ -syn and treated with increasing concentrations of NPT200. Intracellular calcium was assessed using a Fluo-4-AM dye.

## Experimental approach for testing acute NPT200 effect on calcium activity in $\alpha$ -syn Thy-1 tg mice using two-photon microscopy

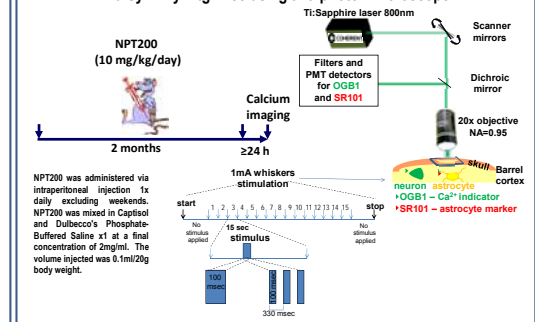


<sup>9</sup>-month old  $\alpha$ -syn tg mice were anesthetized with *o*-chloralose. HR, BP, expired  $\text{CO}_2$  and  $\text{I}$  were continuously monitored. Respiration was aimed to maintain  $\text{pCO}_2$  between 35 and 45 mmHg. A mixture of calcium indicator Oregon Green 488 BAPTA-1 AM (OGB1) and astrocytic marker SR101 were microinjected in the cortical layer 2/3 calcium indicator OGB1. NPT200 (10  $\mu$ M) was dissolved in ACSF and microinjected during imaging. Calcium activity was monitored for 2h post injection. Microinjections were visualized using Alexa Fluor 350 hydrate (blue) added to NPT200 solution.

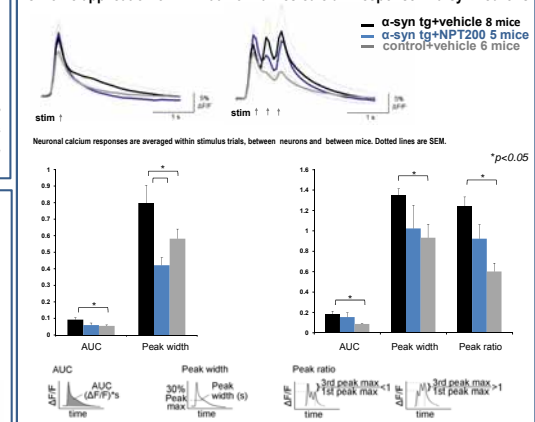
## Acute application of NPT200 normalized calcium response in $\alpha$ -syn neurons



## Experimental approach for testing chronic NPT200 effect on calcium activity in $\alpha$ -syn Thy-1 tg mice using two-photon microscope



## Chronic application of NPT200 normalizes calcium response in $\alpha$ -syn neurons



## Summary & Conclusions:

- NPT200 reversed *in vitro* and *in vivo*  $\alpha$ -syn-related calcium abnormalities.
  - In vitro*: NPT200-induced reductions in intracellular calcium paralleled reductions in  $\alpha$ -syn oligomerization.
  - In vivo acute studies*: NPT200 normalized calcium dynamics in  $\alpha$ -syn Thy1 tg mouse SI cortex.
  - In vivo chronic studies*: NPT200 was most effective in normalizing single neuronal calcium dynamics.
  - These findings in the  $\alpha$ -syn Thy-1 tg mouse model of synucleinopathy suggest a potential therapeutic effect of NPT200.
  - Additional *in vivo* studies are ongoing to determine the optimal treatment regimen required to normalize calcium homeostasis, which may help to elucidate the mechanism of NPT200 action *in vivo*.
- Bibliography:**  
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Rockenstein E, Mallory M, Matthews M, Song S, Crews L, Lansbury Jr (2002) Differential neuroprotective effects of alpha-synuclein in transgenic mice expressing alpha-synuclein from the Parkinson disease genetic locus and Thy-1 promoter. *J Neurosci Res* 68:368-378.  
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