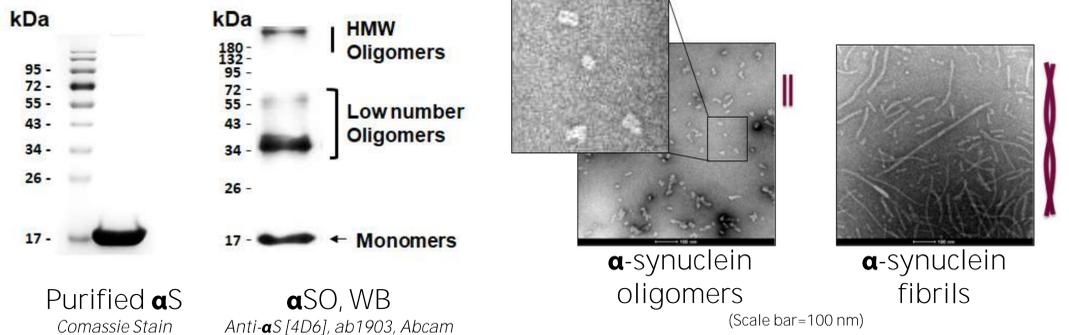


# PATHOLOGICAL ALPHA-SYNUCLEIN PREPARATIONS INDUCE COGNITIVE IMPAIRMENT AND NEURODEGENERATION

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**$\alpha$ -synuclein pathology is clearly linked to Parkinson's disease (PD) and related dementia, which happens early in the disease process. Drug discovery for PD needs translational *in vitro* and *in vivo* models that are recapitulating natural disease onset. Here, we present translational models, induced by minute amount of highly reproducible  $\alpha$ -synuclein oligomers ( $\alpha$ SO) or fibrils ( $\alpha$ SF) for drug screening and discovery.**

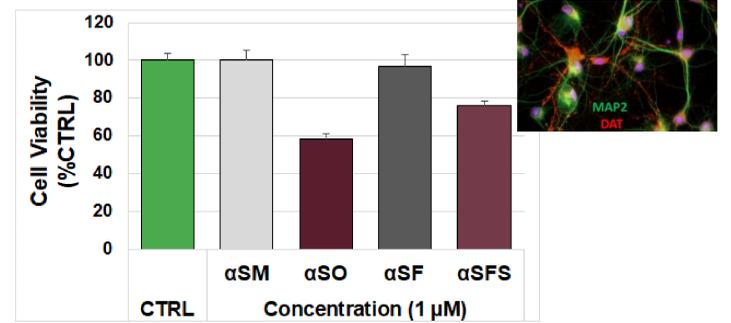
## Characterization of $\alpha$ -Synuclein Aggregates



Recombinant endotoxin-free  $\alpha$ S monomers (>97% purity), detected by Coomassie-stained (left), are treated to generate oligomers ( $\alpha$ SO) or fibrils ( $\alpha$ SF) in a highly reproducible manner.  $\alpha$ SO are stable in SDS-PAGE and can be detected by monomer-directed antibodies (right).

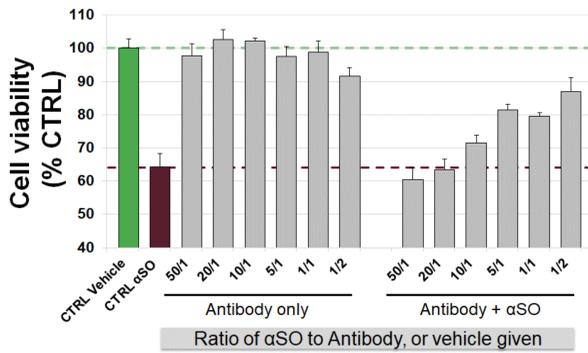
$\alpha$ -Synuclein oligomers, examined by electron microscopy (left), show small duplex shaped forms, different to elongated double helices  $\alpha$ -Synuclein fibrils (right).

## $\alpha$ S-Induced Neurodegeneration in Dopaminergic Primary Neurons



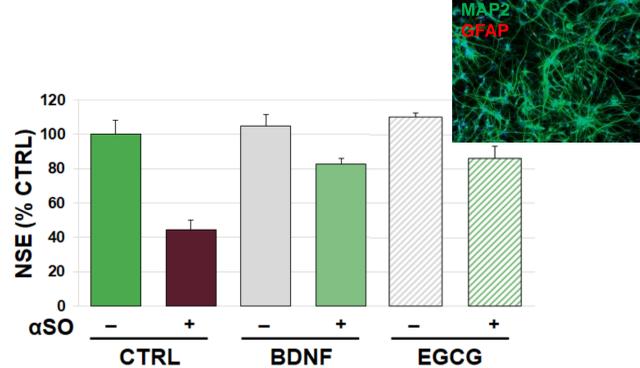
Primary rodent striatal neurons (DIV 10) were incubated for 72 h with 1  $\mu$ M of  $\alpha$ S monomers ( $\alpha$ SM),  $\alpha$ SO, non-sonicated  $\alpha$ SF or sonicated  $\alpha$ S fibrils ( $\alpha$ SFS). Neurons viability (evaluated by the MTT assay) was 60  $\pm$  2.5% and 77  $\pm$  2.5% after  $\alpha$ SO and  $\alpha$ SFS respectively. Neither non-sonicated  $\alpha$ SF nor  $\alpha$ SM were able to induce neurons death (N=3, n = 3).

## Antibodies Prevent $\alpha$ SO- Induced Neurodegeneration



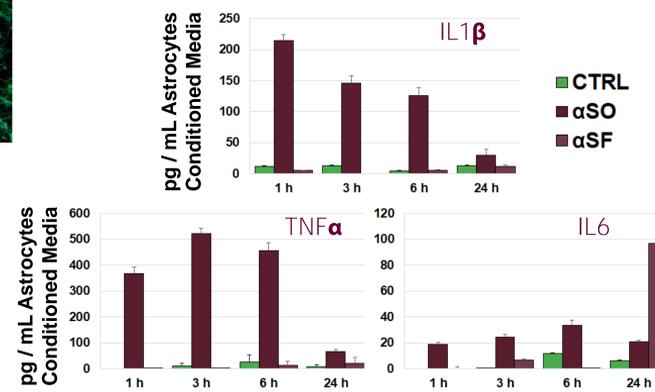
Primary mouse striatal neurons (DIV 10) were incubated for 72 h with vehicle or  $\alpha$ SO and different dilutions of  $\alpha$ -synuclein antibodies. Cell viability was evaluated by the MTT assay.

## $\alpha$ SO Induce Neurodegeneration in Human HIP iPSC-Derived Neurons



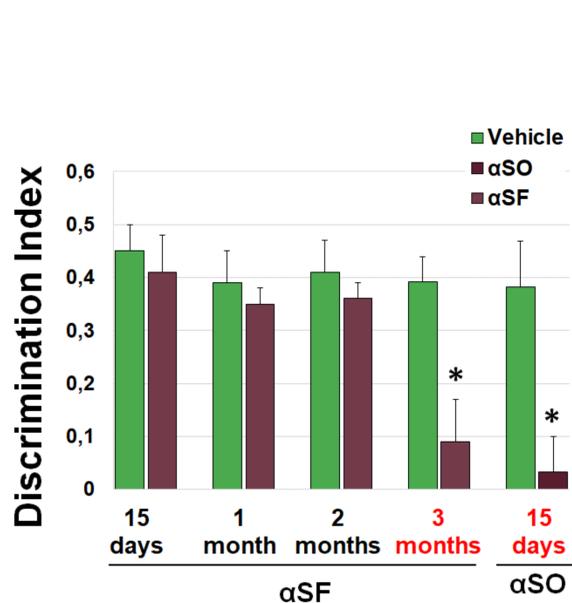
Human hippocampal iPS cells were differentiated for five weeks and treated with 0.3  $\mu$ M  $\alpha$ SO, BDNF, or epigallocatechin gallate (EGCG) for 72 h. Neuronal survival was determined by neuron specific enolase (NSE) ELISA.

## $\alpha$ SO Induce pro-inflammatory cytokines release of primary astrocytes



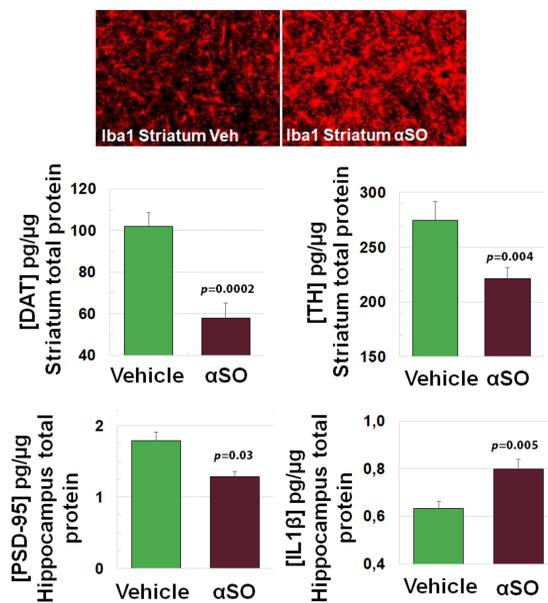
Primary rodent astrocytes were incubated with  $\alpha$ SO or  $\alpha$ SF (10  $\mu$ M) and astrocyte-conditioned media (CM) were harvested at 1, 3, 6 and 24 h post treatment. CM were analyzed by ELISA to quantify pro-inflammatory cytokines levels IL1 $\beta$ , TNF $\alpha$  & IL6 (N=3, n = 2).

## $\alpha$ S Induce Cognitive Impairment in Wild-Type Nontransgenic Mice



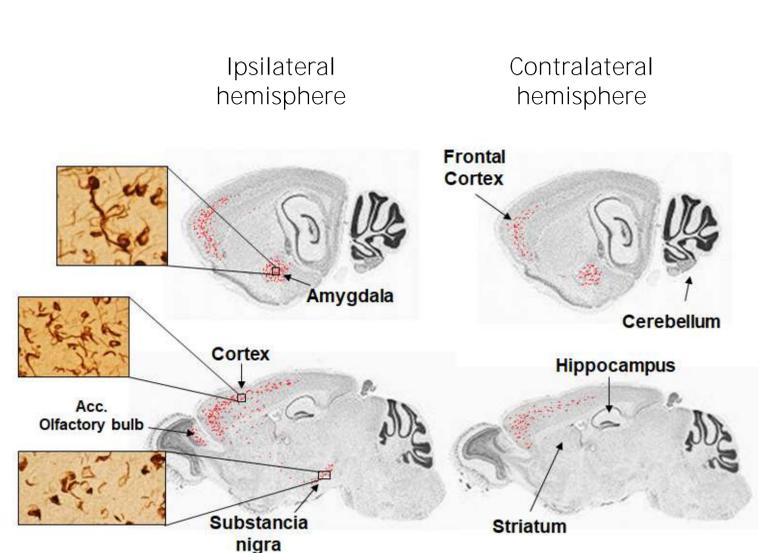
A single intrastriatal inoculation of  $\alpha$ SO or  $\alpha$ SF (4  $\mu$ g) into wild-type mice induced cognitive deficits in the novel object recognition test at different time points.  $\alpha$ SO-induced defects was observed within 15 days and stay the same for up to three months. However,  $\alpha$ SF-induced cognitive dysfunction was not observed before to 3 months. \*  $p$  < 0.05 vs. vehicle

## $\alpha$ SO Induce Dopaminergic Degeneration, Neuro-Inflammation and Synaptic loss



Iba-1 staining showed microglia activation in striatum of  $\alpha$ SO-administered mice. ELISA analysis of mouse striatal lysate showed a significant reduction in dopamine active transporter (DAT) and tyrosine hydroxylase (TH) content in  $\alpha$ SO-inoculated mice. Hippocampal lysate showed increased pro-inflammatory cytokine production (IL1 $\beta$ ) in mice inoculated  $\alpha$ SO, and decreased levels of synaptic markers (PSD-95).

## $\alpha$ SF Induce Spreading in Wild-Type Nontransgenic Mice



A single intrastriatal injection of  $\alpha$ SF (4  $\mu$ g) into wild-type non-transgenic mice led to the cell-to-cell transmission of pathologic  $\alpha$ S and Parkinson's-like Lewy pathology in anatomically interconnected regions. Brain staining for phosphorylated Ser<sub>129</sub> showed spreading of  $\alpha$ S aggregates within 15 days post  $\alpha$ SF intrastriatal administration.