PATHOLOGICAL ALPHA-SYNUCLEIN PREPARATIONS INDUCE COGNITIVE IMPAIRMENT AND NEURODEGENERATION

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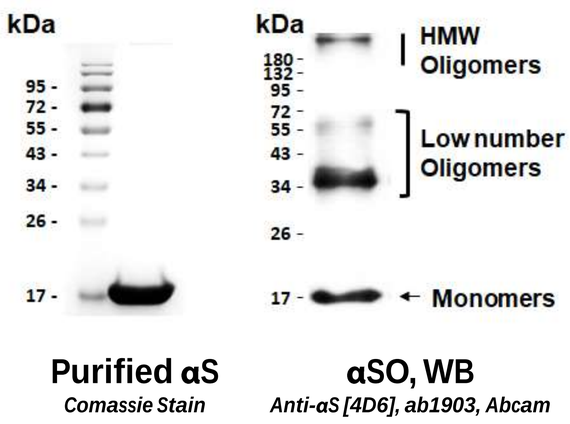
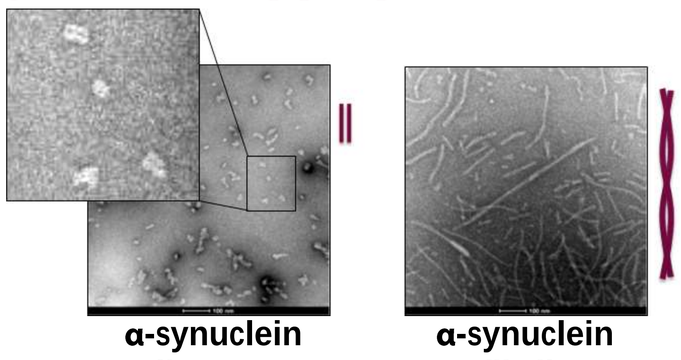
α-synuclein pathology is clearly linked to Parkinson's disease (PD) and related dementia, which happens early in the disease process. Drug discovery for PD needs translational *in vitro* and *in vivo* models that are recapitulating natural disease onset. Here, we present translational models, induced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

Characterization of α-Synuclein Aggregates



Purified αS *Comassie Stain* **αSO, WB** *Anti-αS [4D6], ab1903, Abcam*

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

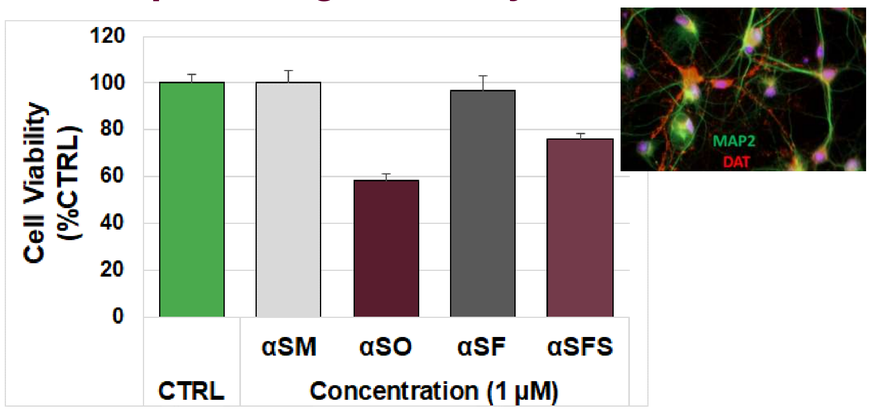


α-synuclein oligomers **α-synuclein fibrils**

(Scale bar=100 nm)

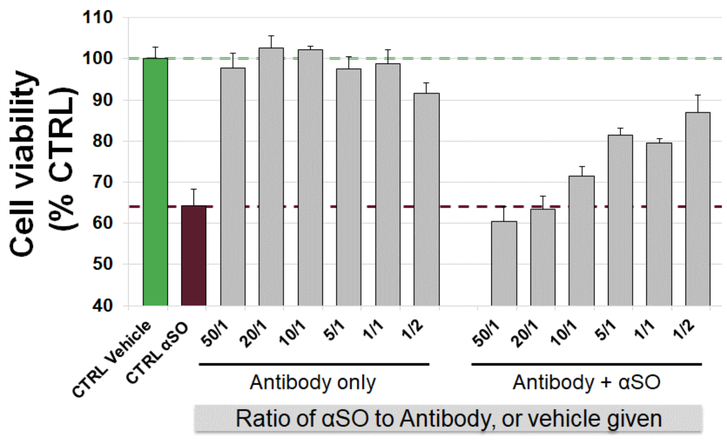
α-Synuclein oligomers, examined by electron microscopy (left), show small duplex shaped forms, different to elongated double helice α-Synuclein fibrils (right).

αS-Induced Neurodegeneration in Dopaminergic Primary Neurons



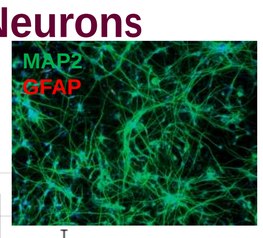
Primary rodent striatal neurons (DIV 10) were incubated for 72 h with 1 µM of αS monomers (αSM), αSO, non-sonicated αSF or sonicated αS fibrils (αSFS). Neurons viability (evaluated by the MTT assay) was 60 ± 2.5% and 77 ± 2.5% after αSO and αSFS respectively. Neither non-sonicated αSF nor αSM were able to induce neurons death (N=3, n = 3).

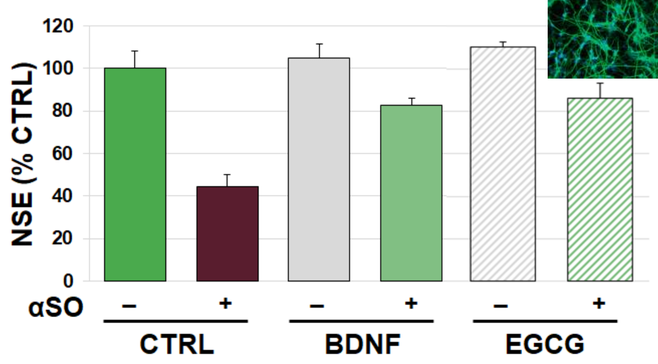
Antibodies Prevent αSO- Induced Neurodegeneration



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

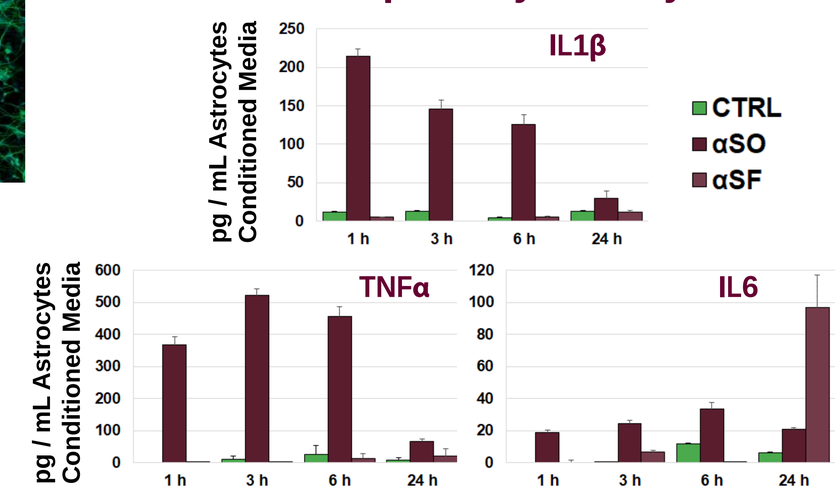
αSO Induce Neurodegeneration in Human HIP iPSC-Derived Neurons





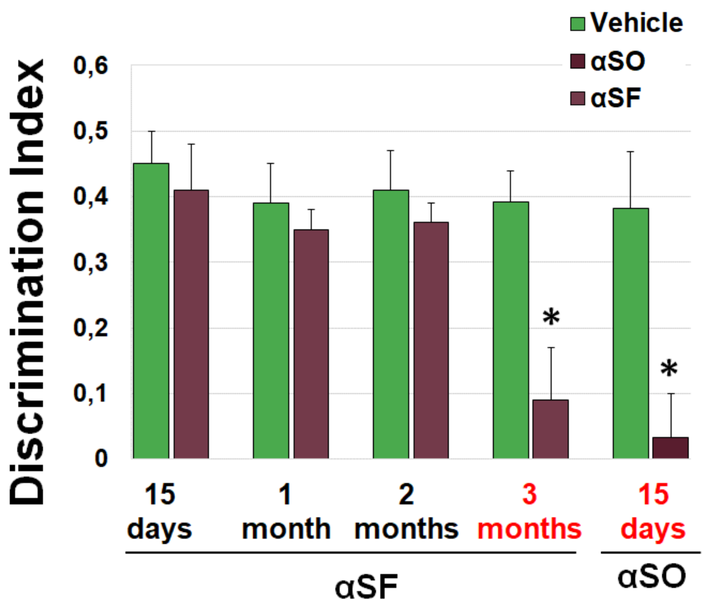
Human hippocampal iPS cells were differentiated for five weeks and treated with 0.3 µM αSO, BDNF, or epigallocatechin gallate (EGCG) for 72 h. Neuronal survival was determined by neuron specific enolase (NSE) ELISA.

αSO Induce pro-inflammatory cytokines release of primary astrocytes



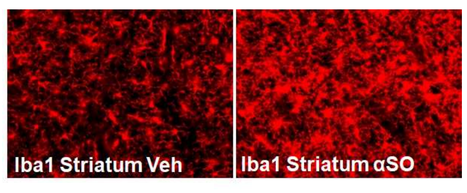
Primary rodent astrocytes were incubated with αSO or αSF (10 µM) and astrocyte-conditioned media (CM) were harvested at 1, 3, 6 and 24 h post treatment. CM were analyzed by ELISA to quantify pro-inflammatory cytokines levels IL1β, TNFα & IL6 (N=3, n = 2).

αS Induce Cognitive Impairment in Wild-Type Nontransgenic Mice



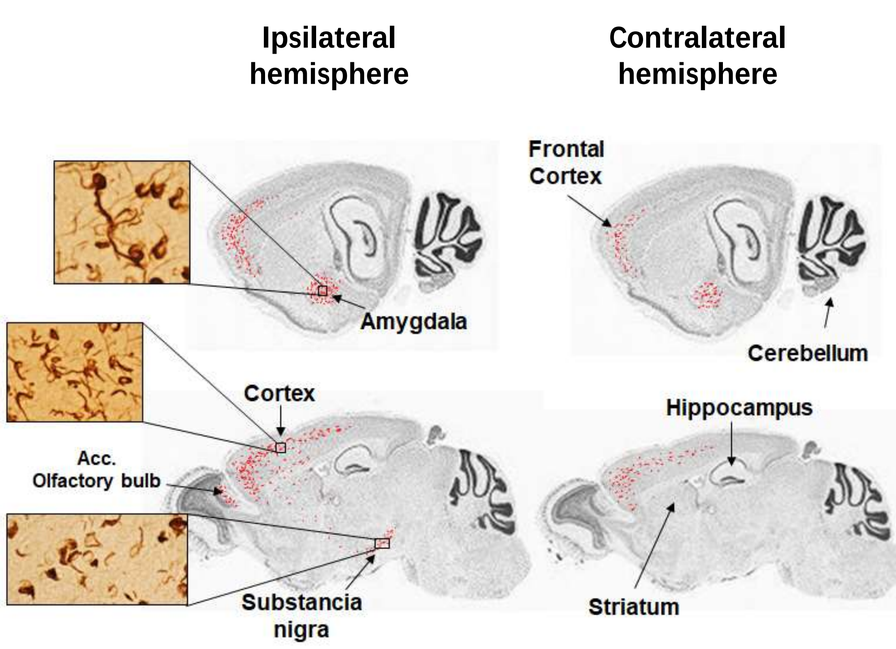
A single intrastriatal inoculation of αSO or αSF (4 µg) into wild-type mice induced cognitive deficits in the novel object recognition test at different time points. αSO-induced defects was observed within 15 days and stay the same for up to three months. However, αSF-induced cognitive dysfunction was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

αSO Induce Dopaminergic Degeneration, Neuro-Inflammation and Synaptic loss



Iba-1 staining showed microglia activation in striatum of αSO-administered mice. ELISA analysis of mouse striatal lysate showed a significant reduction in dopamine active transporter (DAT) and tyrosine hydroxylase (TH) content in αSO-inoculated mice. Hippocampal lysate showed increased pro-inflammatory cytokine production (IL1β) in mice inoculated αSO, and decreased levels of synaptic markers (PSD-95).

αSF Induce Spreading in Wild-Type Nontransgenic Mice



A single intrastriatal injection of αSF (4 µg) into wild-type non-transgenic mice led to the cell-to-cell transmission of pathologic αS and Parkinson's-like Lewy pathology in anatomically interconnected regions. Brain staining for phosphorylated Ser_{129} showed spreading of αS aggregates within 15 days post αSF intrastriatal administration.