Assessing a novel therapy for synucleinopathies using an alpha-synuclein pre-formed fibril mouse model

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Background

What we know about α-synuclein (αsyn):

- Small protein, exists everywhere in the body & abundantly throughout the brain
- Important in neurotransmitter vesicle cycling (1)
- Phosphorylated form (**psyn**) is the primary component of **Lewy bodies (LBs),** the pathological hallmark of synucleinopathies (Fig. 1; 2, 3)

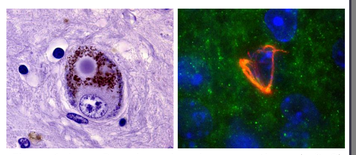


Figure 1: Lewy Body inclusions in human tissue (left, 3) and mouse tissue (right).

What we know about synucleinopathies:

- Second most common form of neurodegeneration
- More common in men
- Includes **Parkinson's disease**, **dementia with Lewy bodies**, & **multiple systems atrophy**
- LB pathology is progressive, spreading to more brain regions over time
- Parkinson's disease presents with motor abnormalities (tremor, slow movement, rigidity, reduced balance and posture) and cognitive/emotional changes (decreased ability to concentrate, depression, anxiety)
- *In vitro* & *in vivo* research shows that αsyn **pre-formed fibrils (PFFs)** leads to the spread of LB inclusions, following the prion-like hypothesis (Fig. 2; 4, 5)
- **Treatments focus on symptom management** – there are no treatments that address neuropathology

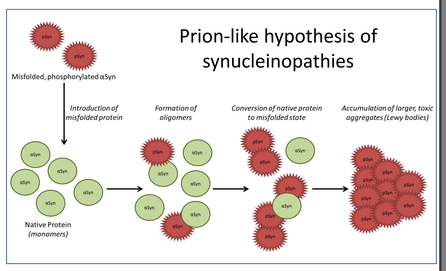


Figure 2: Prion-like hypothesis of synucleinopathies. Introduction of just a small amount of misfolded protein can cause native proteins to misfold and aggregate.

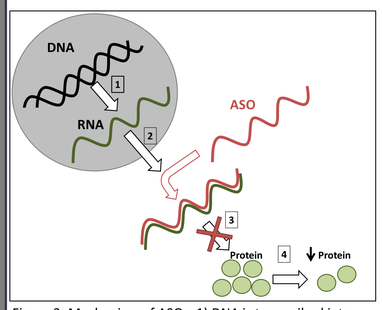


Figure 3: Mechanism of ASOs. 1) DNA is transcribed into RNA. 2) RNA carries the information from DNA to make proteins. 3) Targeted ASOs stop RNA from translating into proteins. 4) Native protein levels decrease

What we know about antisense oligonucleotides (ASOs):

- ASOs prevent the production of specific proteins by inhibiting translation of mRNA (Fig. 3; 6)
- Many neurodegenerative diseases are characterize by accumulation of certain misfolded proteins
- Thus, **ASOs have the potential to slow or reverse disease progression by decreasing protein available to be converted into toxic aggregates**

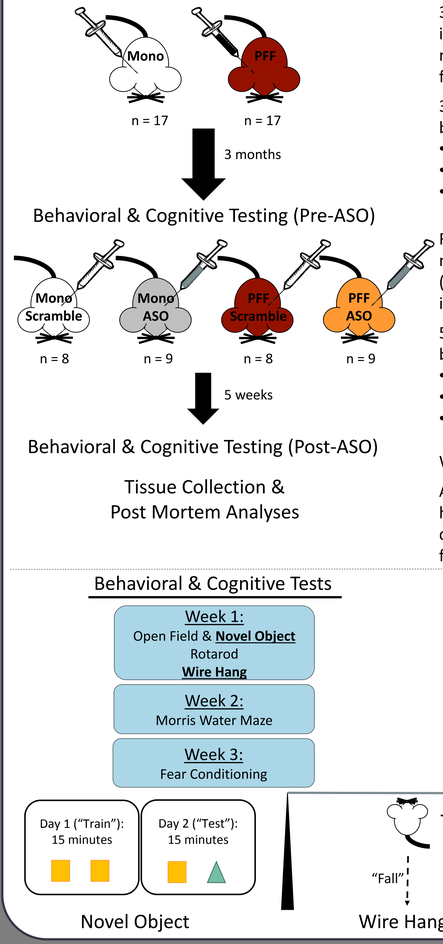
Goal

Assess the therapeutic efficacy of a novel, αsyn-targeted ASO

Hypothesis

Treating PFF-injected mice with an α-synuclein specific antisense oligonucleotide will reduce pathology and rescue behavioral and cognitive decline

Materials & Methods



34 male C57Bl/6J (wild-type) received bilateral injections into the motor cortex of either 5ug of monomeric αsyn ("**Mono**") or 5ug αsyn pre-formed fibrils ("**PFF**").

3 months later, mice received a battery of behavioral tests to examine

- motor function (rotarod, wire hang)
- anxiety-like behavior (open field), and
- cognitive performance (novel object, water maze, fear conditioning).

Following behavior, mice received 700ug of either a nonsense ASO ("**Scramble**") or an αsyn-specific ASO ("**ASO**"), delivered via an intracerebroventricular injection.

5 weeks later, mice received another battery of behavioral tests to assess the effects of the ASO on

- motor function (rotarod, wire hang),
- anxiety-like behavior (open field), and
- cognitive performance (novel object, water maze).

Weekly body weights were recorded to track health.

After behavioral assessment of ASO, the right hippocampus, cortex, and cerebellum were dissected and frozen; the left hemisphere was post-fixed; and intestine and plasma were collected.

Behavioral & Cognitive Tests

- Week 1: Open Field & **Novel Object** Rotarod **Wire Hang**
- Week 2: Morris Water Maze
- Week 3: Fear Conditioning

Post Mortem Analyses

- **Western Blots** (protein levels)
- Immunohistochemistry (pathology spread)
- Gut Microbiome (diversity & inflammation)

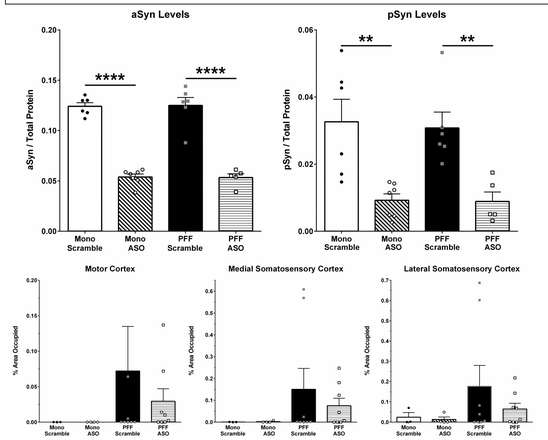
Summary

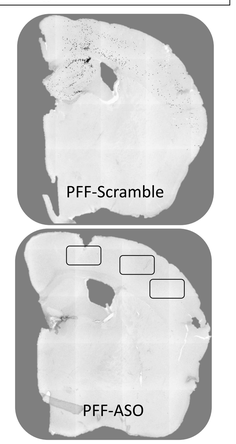
- The αsyn-targeted ASO successfully reduced αsyn and psyn.
- However, αsyn reduction caused severe weight loss, decreased food intake, and detriments in motor and cognitive function.
- This suggests αsyn has a vital function in the adult brain that is still unknown and needs to be further explored.

Results

ASO reduces αsyn & pathology

The αsyn-ASO successfully reduced amount of αsyn and psyn protein in the hippocampus; it also appears to have mildly alleviated LB-like pathology

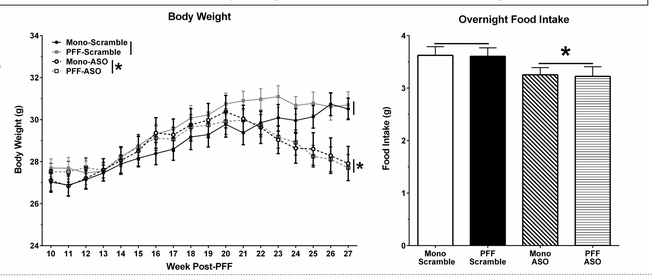




Reduction of αsyn causes weight loss

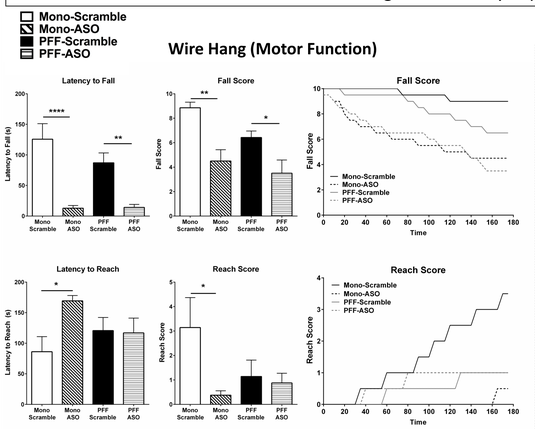
ASO-treated mice showed a decline in body weight and decrease in overnight food intake

Treatment with ASO caused a dramatic and continuous drop in body weight. ASO also reduced overnight food intake, suggesting that αsyn in the hypothalamus is important for regulation of typical body functions.

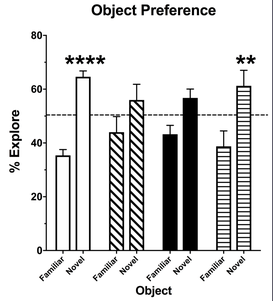


Reduction of αsyn impairs motor function & cognitive abilities

Mice treated with ASO showed impaired motor functions across behavioral tests and alterations in learning and memory capabilities



ASO caused animals to fall off the wire hang more, regardless of PFF-status. In monomeric animals, ASO impeded their ability to coordinate their movements to climb; in PFF-animals, reaching was impaired regardless of which ASO treatment they received.



Monomeric animals that received ASO did not prefer the novel object; conversely, ASO improved novel object recognition in PFF-injected mice.

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