

New *in vivo* model of Parkinson's disease involving combined toxicity of alpha-synuclein oligomers and protofibrils, and chronic inhibition of GBA.



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Introduction

Parkinson's disease is usually diagnosed at 60 years of age and symptoms include resting tremor and bradykinesia, caused by the degeneration of the nigrostriatal dopaminergic pathways. We know that accumulation of misfolded alpha-synuclein (α -syn), forming lewy-bodies, causes mitochondrial stress and impairs autophagy lysosomal pathway in Parkinson's disease. Furthermore, mutations on the gene coding for the lysosomal protein GBA is a strong risk factor for Parkinson's disease. Preclinical models of Parkinson's disease often rely on one insult despite the multifactorial pathophysiology of this disease. Here, we developed and characterized a novel *in vitro* model of Parkinson's disease, based on α -syn toxicity and GBA-linked lysosomal dysfunctions.

Methods

Animals:

18-month-old C57BL/6J mice were used in this model. All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and followed current European Union regulations (Directive 2010/63/EU).

Surgery and treatment:

Stereotaxic injections of α -syn protofibrils: Human α -syn peptide preparation containing protofibrils precisely evaluated by western blot. Mice injected into the SNpc either 2.5 μ L of vehicle (NaCl, 0.9 %) or 2.5 μ L of α -syn preparation bilaterally.

CBE administration: CBE was ip administered, starting the day of the stereotaxic injections of α -syn. CBE was given three times per week at 50 mg/kg.

Behavioral testing:

To detect motor dysfunctions related to Parkinson's disease, we used the grid walking test and the bar test.

Immunostaining:

Brains were perfused with PBS and PFA 4 %. 40 μ m free-floating sections were incubated with antibodies. Images were acquired with a confocal laser-scanning microscopy. MetaXpress[®] (Molecular Devices) software was used for automated picture analysis.

Conclusions

- SNpc injections of α -syn protofibrils combined with a chronic inhibition of GBA, led to histological phenotype highly similar to those observed in patients.
- α -syn/CBE reproduces the causes of Parkinson's disease, the symptoms of the disease and respond to symptomatic medication given to patients.
- This animal model is therefore a valuable tool for studying the pathophysiology of Parkinson's disease and test drug candidates.

Results

Intra-nigral injection of α -syn and chronic inhibition of GBA cause a loss of dopaminergic neurons in aged mice

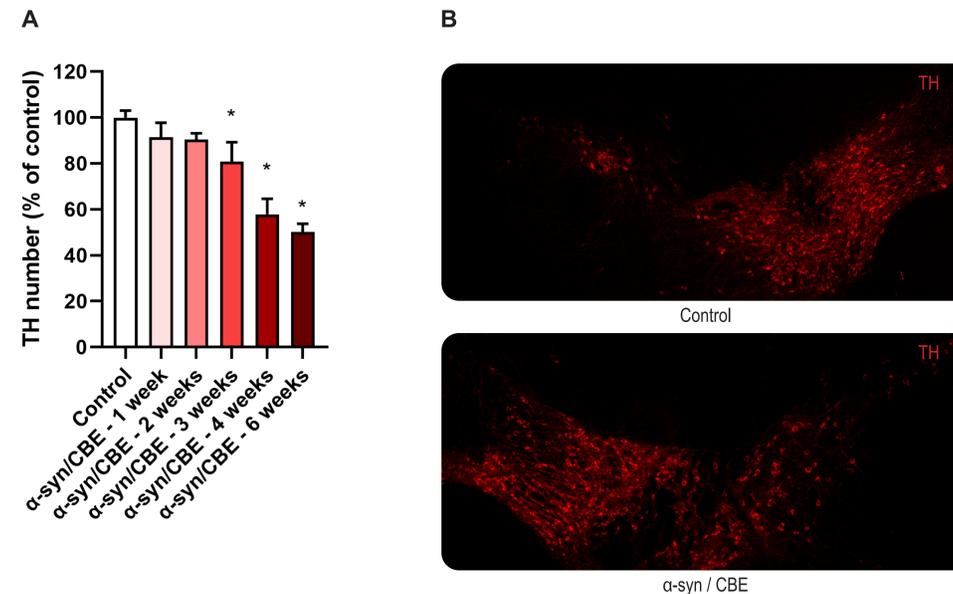


Figure 1: Kinetic of neurodegeneration. (A) Progressive loss of TH(+) dopaminergic neurons after intra-nigral α -syn injections and GBA inhibition (α -syn/CBE). (B) Representative pictures of TH(+) cells in the substantia nigra pars compacta.

Intra-nigral injection of α -syn and chronic inhibition of GBA cause a loss of dopaminergic neurons in aged mice

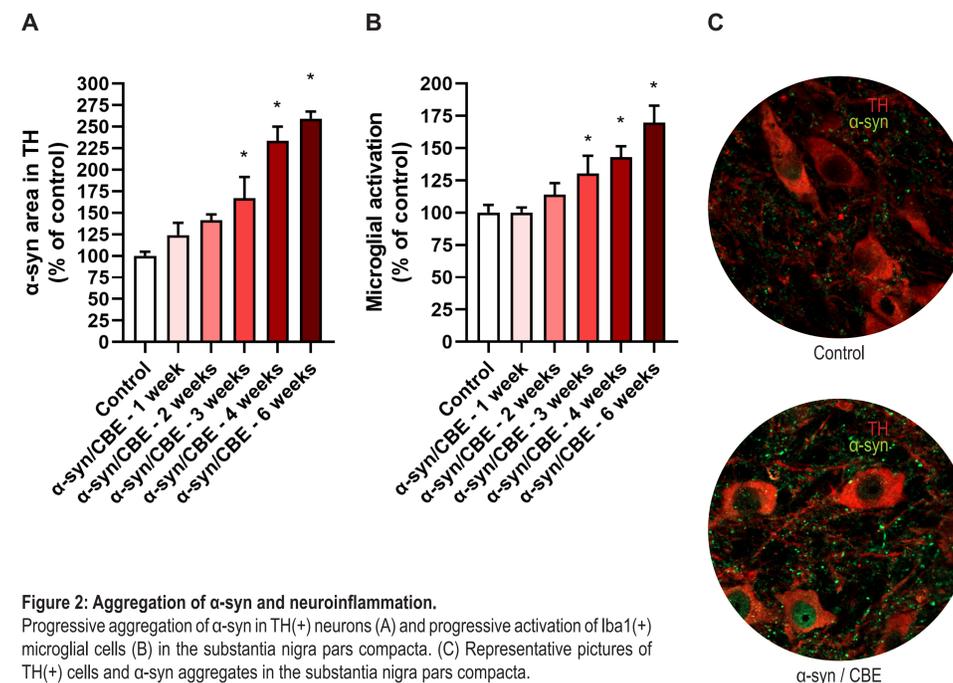


Figure 2: Aggregation of α -syn and neuroinflammation. Progressive aggregation of α -syn in TH(+) neurons (A) and progressive activation of Iba1(+) microglial cells (B) in the substantia nigra pars compacta. (C) Representative pictures of TH(+) cells and α -syn aggregates in the substantia nigra pars compacta.

Mitochondrial stress and impaired autolysosomal pathway in dopaminergic neurons of α -syn/CBE mice

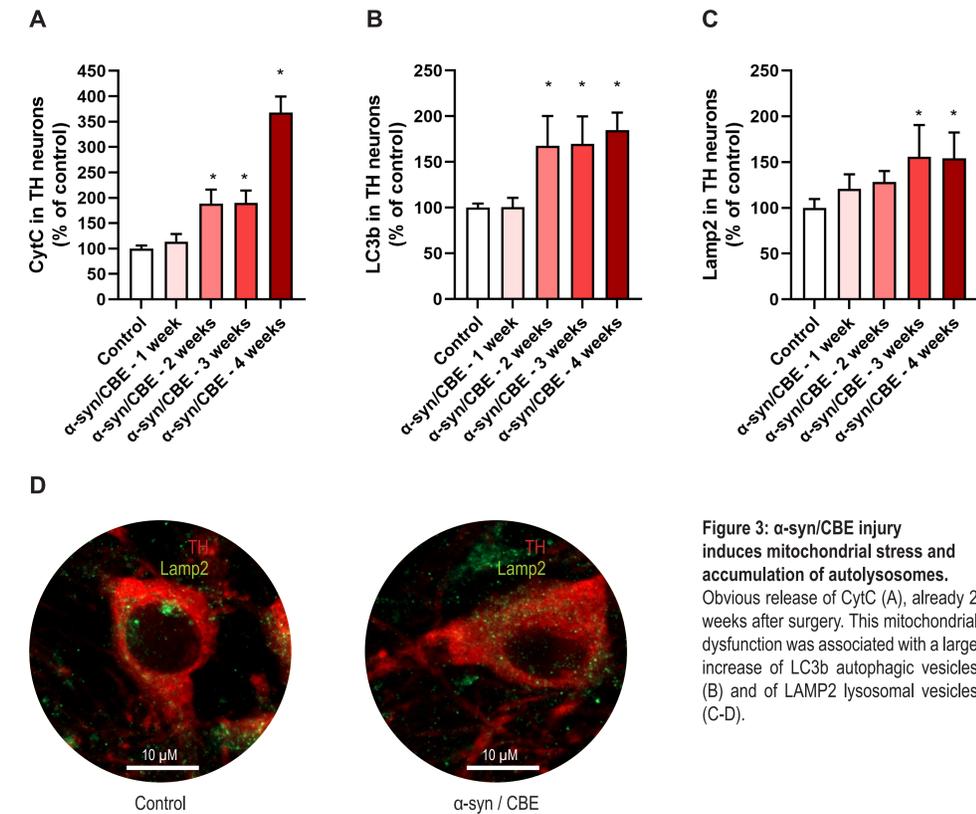


Figure 3: α -syn/CBE injury induces mitochondrial stress and accumulation of autolysosomes. Obvious release of CytC (A), already 2 weeks after surgery. This mitochondrial dysfunction was associated with a large increase of LC3b autophagic vesicles (B) and of LAMP2 lysosomal vesicles (C-D).

Intra-nigral injection of α -syn and chronic inhibition of GBA induce motor dysfunction

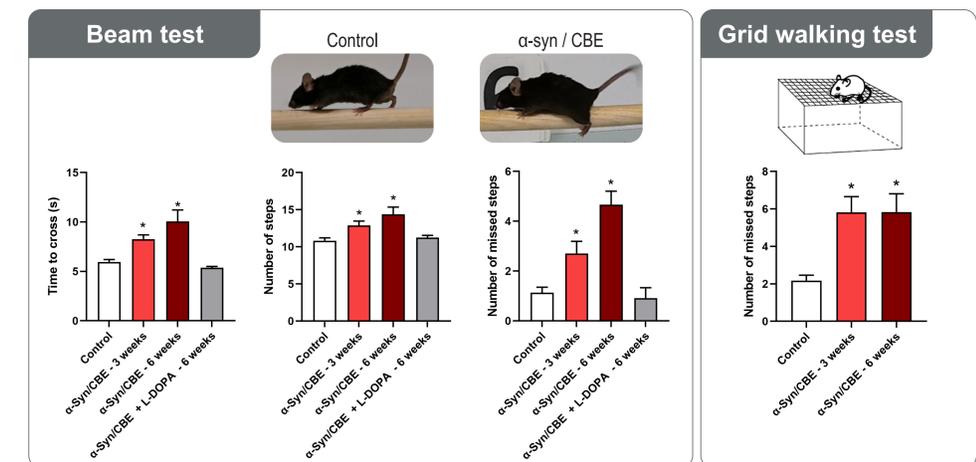


Figure 4: α -syn/CBE mice are characterized by motor deficit. (A-C) On beam test, α -syn/CBE mice were slower, made more steps to reach the platform and presented with a higher number of slips compared to control mice. These motor dysfunctions were still observed 6 weeks after surgery. L-Dopa, a DA precursor, improved motor coordination of α -syn/CBE mice. (D) On grid walking test, 3 weeks after the surgery, α -syn/CBE mice showed an increased number of missed steps compared to the control mice. Similar observation was seen 6 weeks after the surgery, supporting the fact of motor deficit was maintained over the time.