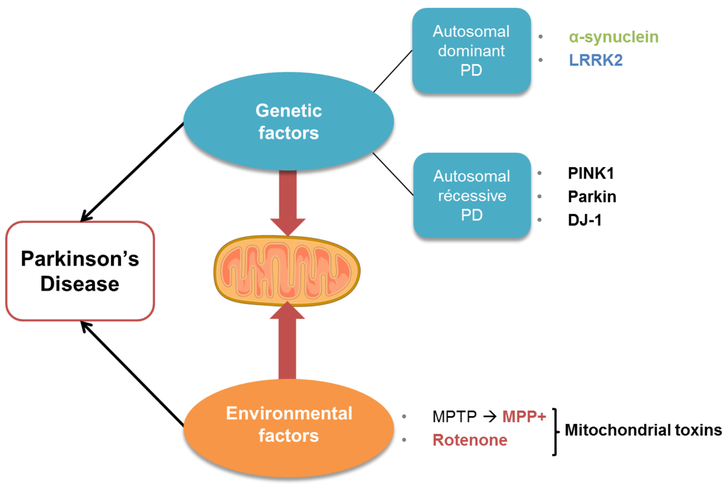
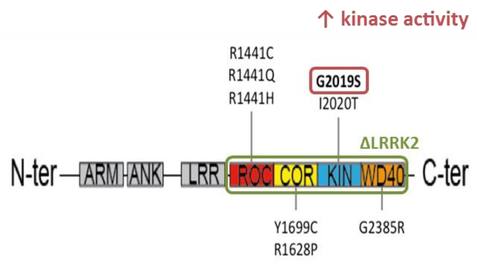
Alpha-synuclein and LRRK2's cooperation in **mitochondrial dysfunctions** in Parkinson's Disease (PD)

Camille Gardier, Noémie Cresto, Marie-Claude Gaillard, Noëlle Dufour, Alexis Bemelmans, Emmanuel Brouillet & Géraldine Liot
Laboratoire des Maladies Neurodégénératives – CNRS UMR 9199



PD is the second most frequent neurodegenerative disease after Alzheimer's Disease. It is characterized by the selective loss of dopaminergic (DA) neurons in the SNpc and the formation of Lewy Bodies, composed by aggregates of **α-synuclein** (αsyn). The mutation A53T of this protein can accelerate its aggregation. The pathogenesis of PD is not really understood but it results from a complex interaction between genetic and environmental factors, and many clues point towards a huge involvement of mitochondrial alterations.

LRRK2 (Leucine-Rich Repeat Kinase 2) is a protein that possesses both a kinase and a GTPase domain. It is involved in many cellular processes, such as autophagy and microtubules and neurite growth. The G2019S mutation in its kinase domain – responsible for an increased kinase activity – is the most common cause of autosomal dominant forms of PD, but is also found in some sporadic cases (Martin *et al*, 2014). We work with a truncated form of LRRK2 = ΔLRRK2, composed of the Cter part of the protein, containing the GTPase and kinase domains.

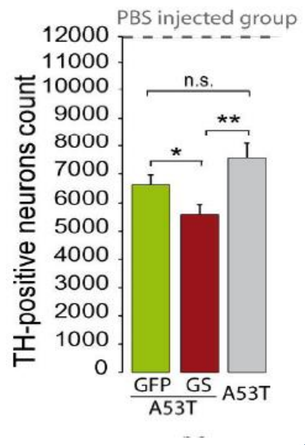


Preliminary results (Noémie Cresto, PhD)

15 weeks after the stereotaxic co-injection of an AVV coding for **ΔLRRK2^{G2019S}** and an AAV coding for **αsyn^{A53T}** in the SNpc of rats, a significant decrease of the number of DA neurons was observed compared to the injection of AAV-αsyn^{A53T} alone, suggesting a functional interaction between LRRK2 and αsyn.

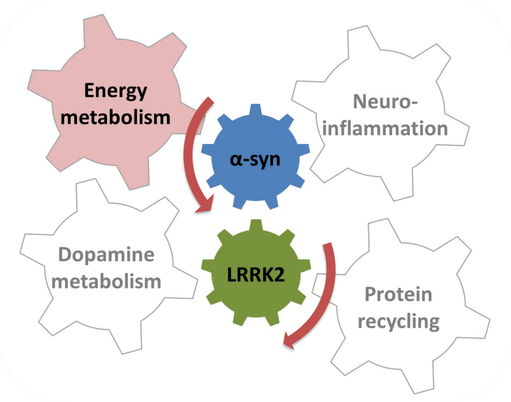
Our aim is therefore to try to decipher the mechanism of this functional interaction, focusing on the mitochondrial mechanism.

Separately, both LRRK2 and αsyn have been shown to localize to mitochondria and to alter mitochondrial function. Mitochondria is extensively studied in PD, initially because of the post-mortem description of a complex I deficiency in the striatum (Mizuno et al. 1989) and in the substantia nigra (Schapira et al. 1990) of PD patients. It is also known that environmental factors inhibiting this mitochondrial complex I (for example rotenone or MPTP) can induce parkinsonian symptoms (Langston and Ballard 1983; Schapira 2008).



→ Mitochondrial hypothesis for the functional interaction of LRRK2 and αsyn

Hypothesis



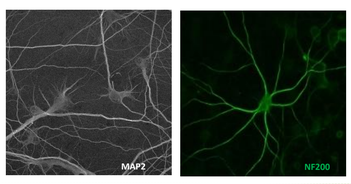
LRRK2 could directly or indirectly worsen αsyn-induced mitochondrial deficits.

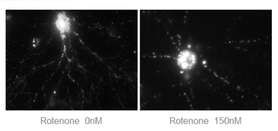
Methods – screening of mitochondrial function

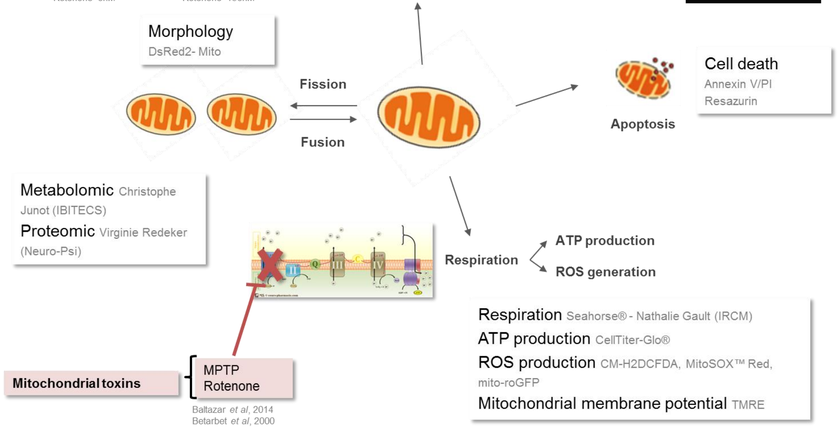
Rat primary cortical neurons

- Lenti-ΔLRRK2$^{WT/G2019S/DK}$
- Lenti-α-syn$^{WT/A53T}$

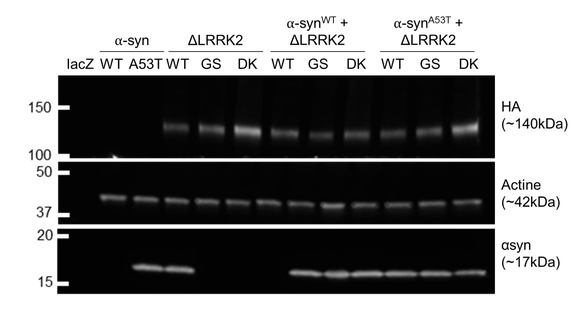
} +/- mitochondrial toxins

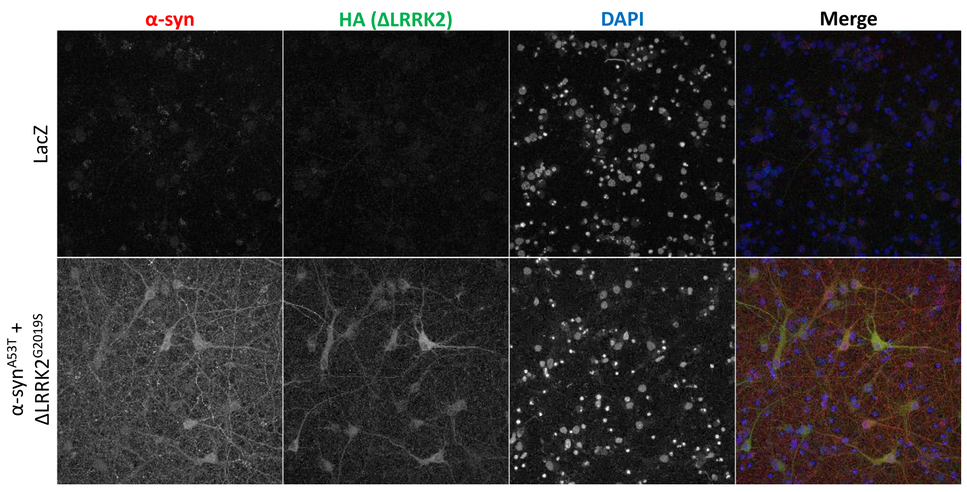






Co-expression of ΔLRRK2 and αsyn

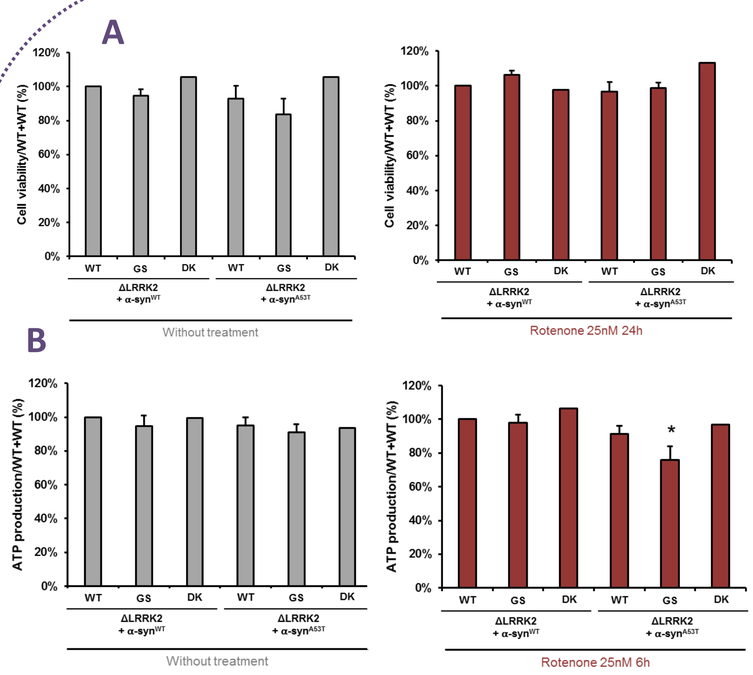




Primary rat cortical cultures were obtained by dissecting the cortices of E17 rat embryos. At DIV1, the cells were infected with lentivirus coding for lacZ or ΔLRRK2$^{WT/G2019S/DK}$ (20ng/100.000cells) and αsyn$^{WT/A53T}$ (10ng/100.000cells). At DIV14, the cells where either **A**. lysed in a triton buffer to perform a Western-Blot analysis of the expression levels of αsyn and ΔLRRK2, or **B**. fixed in formaldehyde 3,7% + 5% sucrose and a double-stained for αsyn and HA (for ΔLRRK2) (magnification 40X)

→ Validation of the cellular model of co-expression of LRRK2 and αsyn

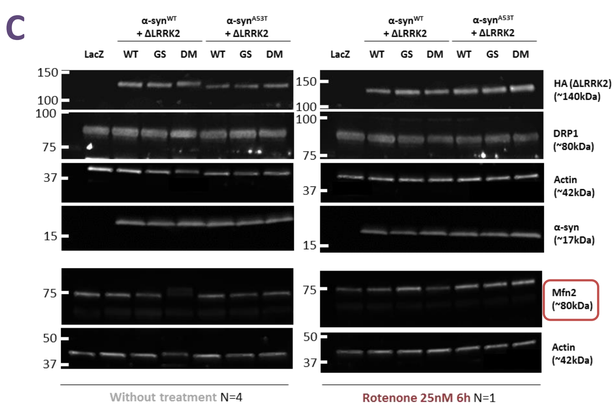
Results : screening of mitochondrial function



Primary rat cortical cultures were obtained by dissecting the cortices of E17 rat embryos. At DIV1, the cells were infected with lentivirus coding for ΔLRRK2$^{WT/G2019S/DK}$ (20ng/100.000cells) and αsyn$^{WT/A53T}$ (10ng/100.000cells). At DIV14, cells where treated or not with 25nM of rotenone for 6h. N=1-4, n=5.

A. Cellular viability was evaluated by a resazurine test. The level of fluorescence was measured with a spectrofluorometer. The results are presented as the proportion of live cells compared to the α-syn^{WT} + ΔLRRK2^{WT} condition (mean + SEM).

B. ATP production was evaluated using the CellTiter-Glo 2.0 Assay (Promega). The level of luminescence was measured with a luminometer. The results are presented as the percentage of ATP produced compared to the α-syn^{WT} + ΔLRRK2^{WT} condition (mean + SEM). *p<0,05, ANOVA and Scheffe post hoc compared to WT+WT.



C. A WesternBlot Analysis of cell lysates was conducted to evaluate the expression level of Mitofusin 2 (implicated in mitochondrial fusion events) and DRP1 (implicated in mitochondrial fission events). N=4 without treatment / N=1 with rotenone.

→ ΔLRRK2^{G2019S} seems to increase α-syn^{A53T}'s mitochondrial toxicity

Perspectives

- [x] Setting up a new *in vitro* model to study the mitochondrial mechanisms of the interaction between **ΔLRRK2** and **αsyn**.

Setting up the treatments to sensitize neurons to **ΔLRRK2** and **αsyn**:

- [x] Acute drug treatment
- [ ] Chronic drug treatment
- [x] Medium without antioxidants

Screening mitochondrial function:

- [x] Cell death
- [ ] Energy metabolism
- [ ] Mitochondrial traffic/morphology

Identifying new targets:

- [ ] Proteomic studies
- [ ] Metabolomic studies

Our first results indicate that ΔLRRK2^{G2019S} modulates α-syn^{A53T}'s mitochondrial toxicity in our *in vitro* model

References

• Langston, J. W., & Ballard, P. A. (1983). Parkinson's disease in a chemist working with 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine. *The New England Journal of Medicine*, *309*(5), 310
• Martin, I., Kim, J. W., Dawson, V. L., & Dawson, T. M. (2014). LRRK2 pathobiology in Parkinson's disease. *Journal of Neurochemistry*.
• Mizuno, Y., Ohta, S., Tanaka, M., Takamiya, S., Suzuki, K., Sato, T., … Kagawa, Y. (1989). Deficiencies in Complex I subunits of the respiratory chain in Parkinson's disease. Biochemical and Biophysical Research Communications, 163(3), 1450–1455.
• Schapira, a H., Cooper, J. M., Dexter, D., Clark, J. B., Jenner, P., & Marsden, C. D. (1990). Mitochondrial complex I deficiency in Parkinson's disease. Journal of Neurochemistry, 54(3), 823–827.
• Schapira, A. H. (2008). Mitochondria in the aetiology and pathogenesis of Parkinson's disease. The Lancet Neurology.