

Ph.D. Program in Molecular, Cellular and Environmental Biology
40th Cycle - Curriculum: Molecular and Cellular Biology

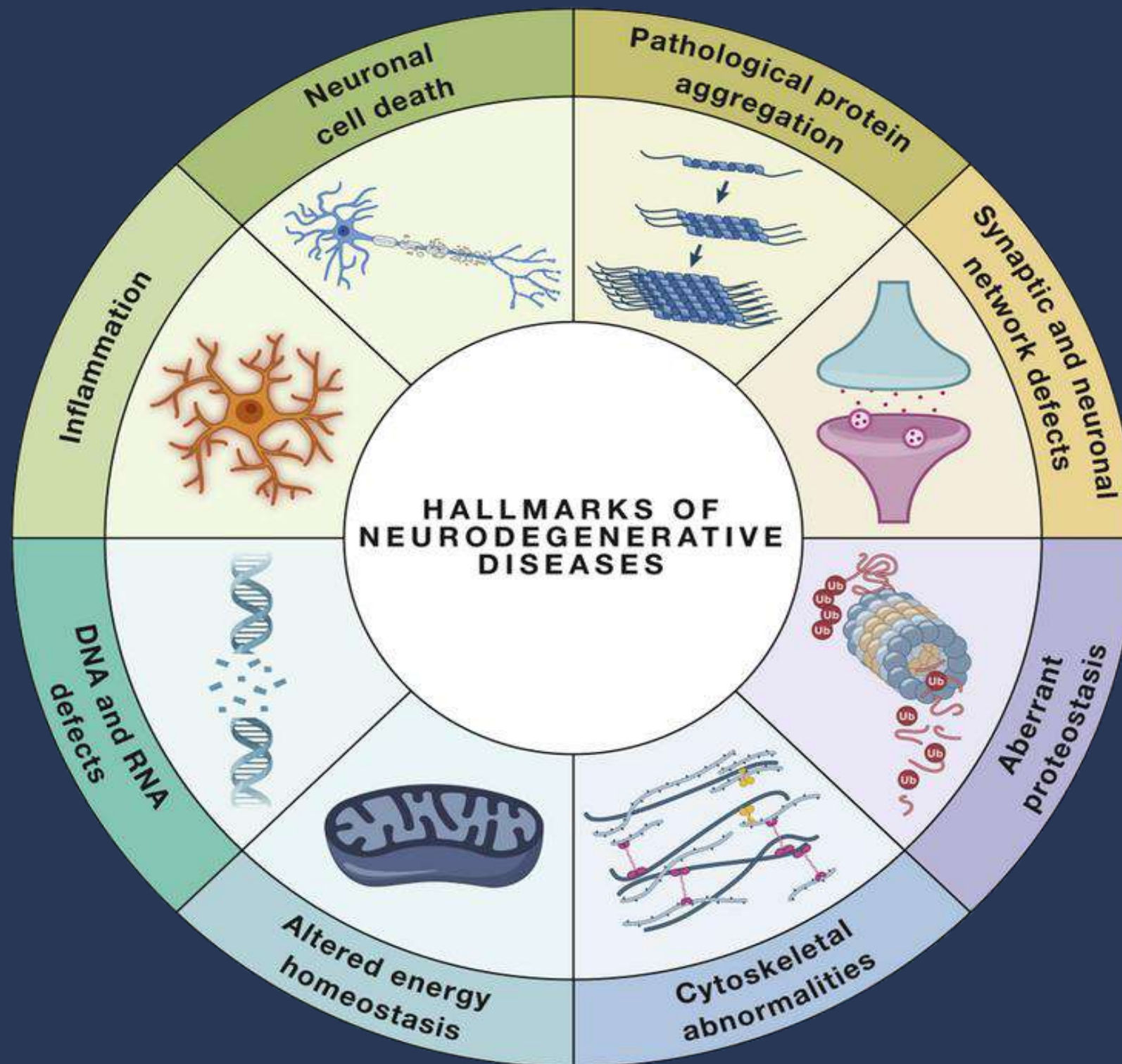
**Morphological, ultrastructural and molecular study of neurodegeneration
using innovative digital imaging procedures**

TUTOR:
Prof.ssa Sandra Moreno

CANDIDATE:
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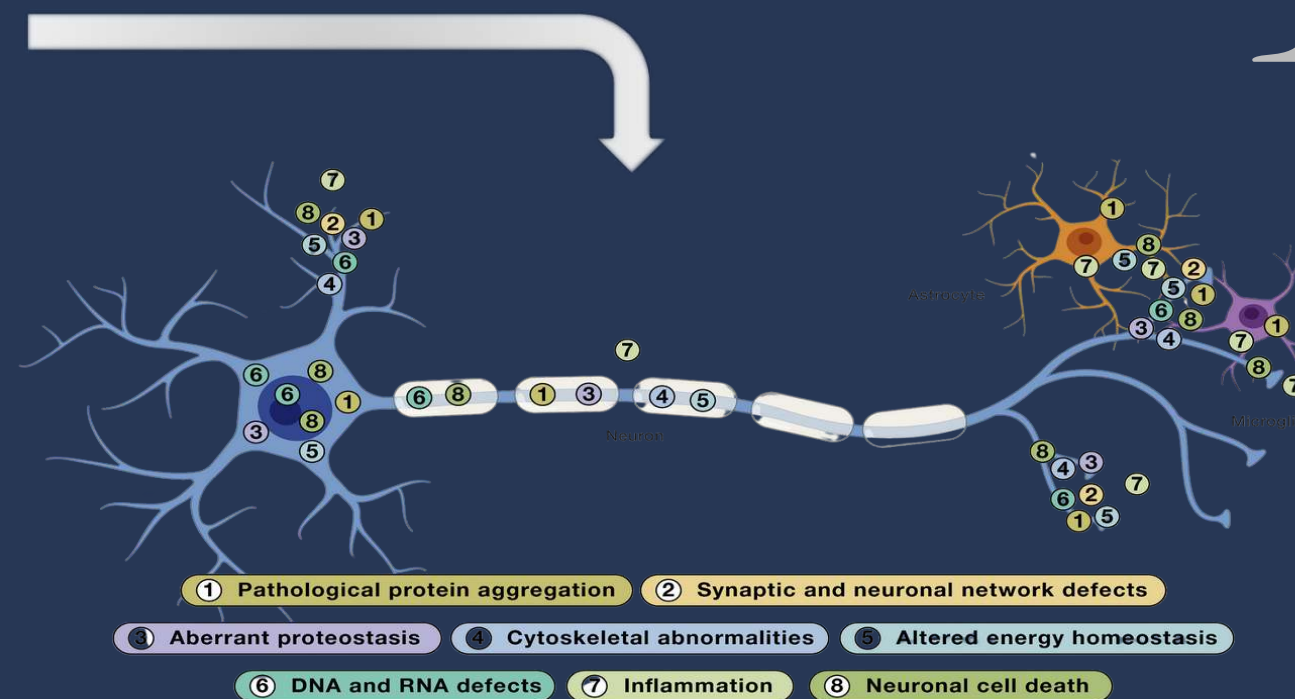
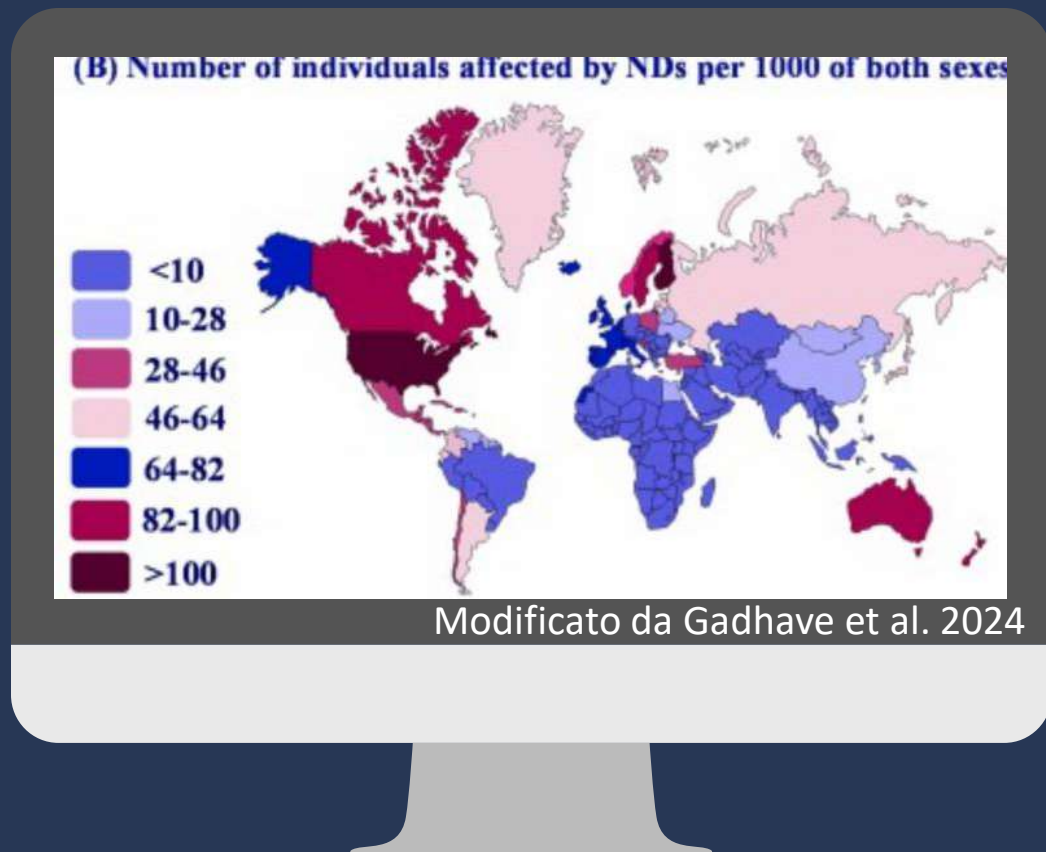
NEURODEGENERATIVE DISEASES

Neurodegenerative disorders are characterized by progressive loss of neuronal function and structure culminating in cell death.



Prevalence of most common neurodegenerative diseases:

1. Alzheimer's Disease (83,6%)
2. Parkinson's Disease (12,3%)
3. Multiple Sclerosis (2,6%)
4. Huntington's Disease (0.6%)
5. ALS (0,1%)



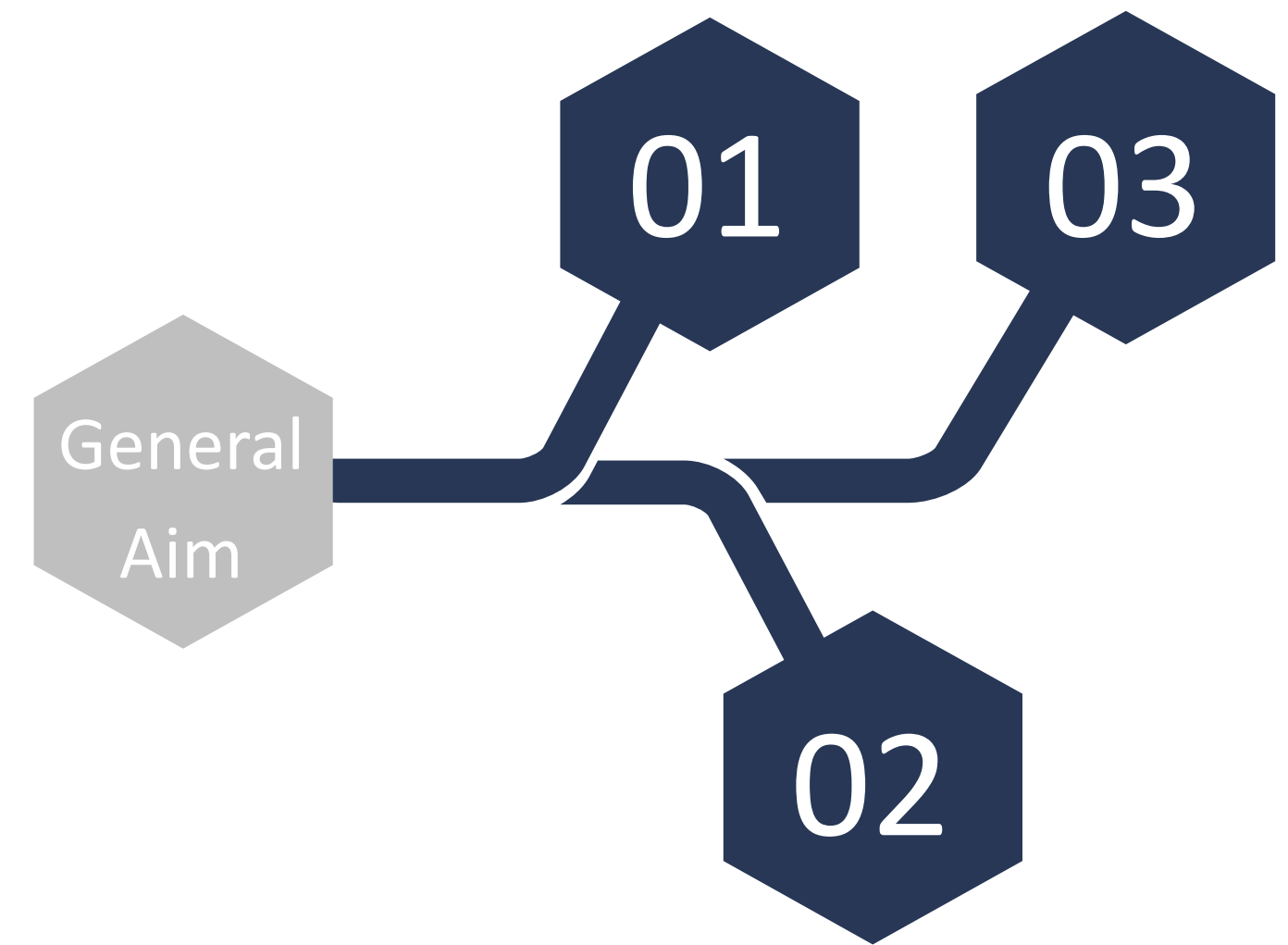
General Aim:

This project aims to investigate and characterize the involvement of peroxisomes in cell survival and death processes in various neurodegeneration models using specific confocal, electron, and focused ion beam microscopy procedures.

This integrated approach will provide a more comprehensive and detailed view of the structural, ultrastructural, and functional changes occurring in neural cells during neurodegeneration.

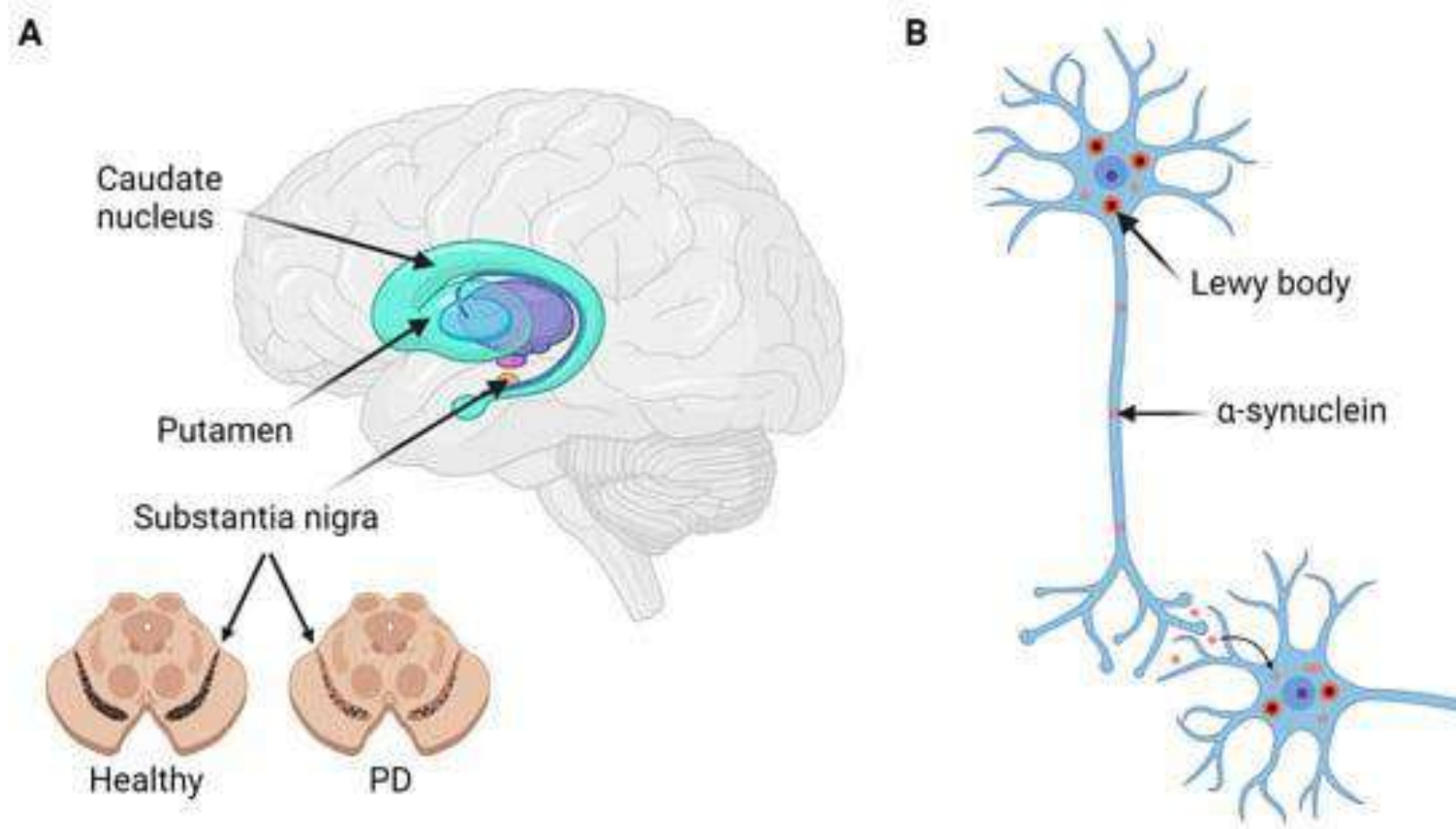
Specific Aims:

- 01 Investigating the role of peroxisomes in AD and PD
- 02 Evaluating the cross-talk mitochondria-peroxisomes
- 03 Explore potential neuroprotective effects of PPAR- α agonists



PARKINSON'S DISEASE (PD)

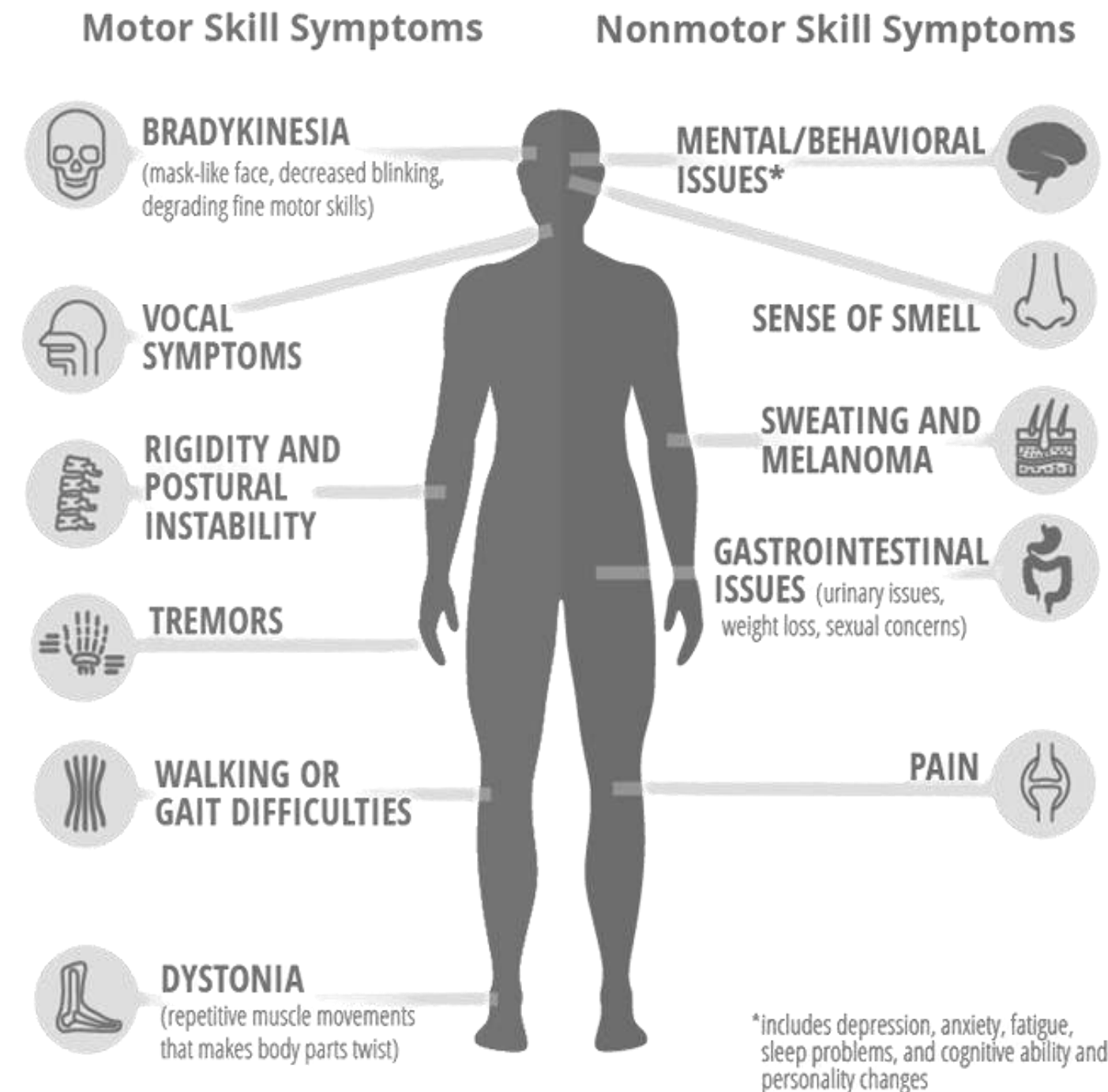
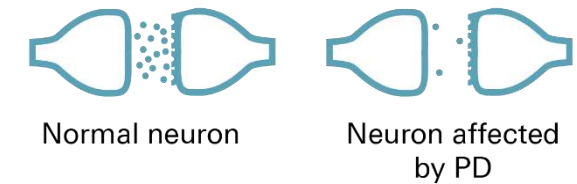
Klokkaris et al., 2024



RISK FACTORS

- **Age:** Advanced age is the most significant factor associated with PD. The risk of developing this condition increases over 60 years of age.
- **Genetics:** Certain genetic mutations and variations have been linked to risk of PD.
- **Family history:** Having a close relative, including a parent or sibling, with PD increases chances of developing the condition.
- **Environmental Factors:** Exposure to environmental factors has been associated with PD. Such factors include pesticides, herbicides, industrial chemicals, solvents and heavy metals.
- **Sex:** Biological men have a higher risk of developing Parkinson's disease compared to biological women.

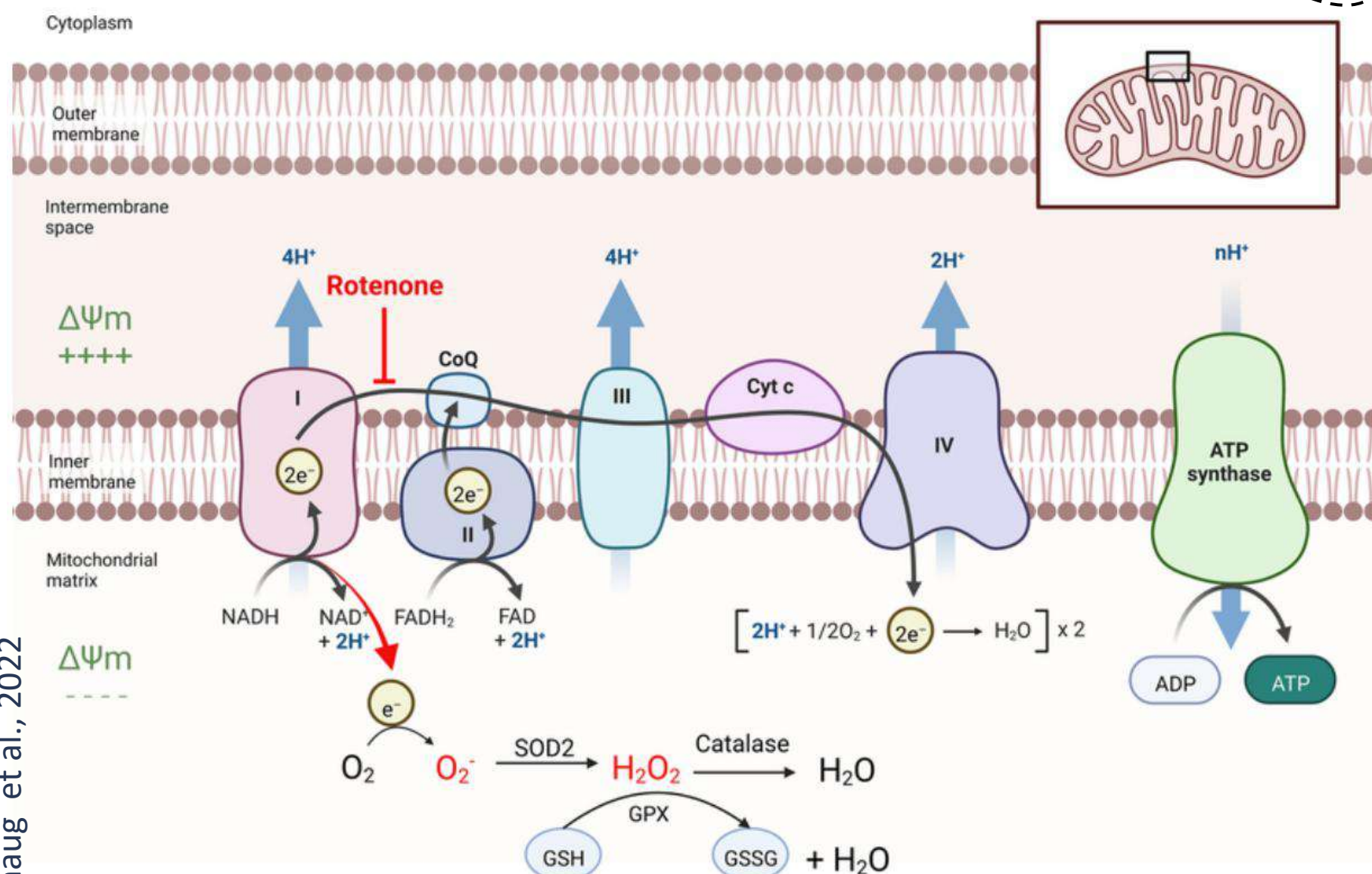
- 2nd most common progressive neurodegenerative disorder
- Degeneration/loss of dopaminergic neurons in the *substantia nigra pars compacta* of the midbrain
- Formation of neuronal **Lewy's Bodies** (aggregates of α -synuclein)



- Rotenone is a highly lipophilic isoflavonoid found in the Fabaceae family and is used as a broad-spectrum natural pesticide.
- The World Health Organization (WHO) currently classifies rotenone as a Class II, moderately hazardous, pesticide.

The best-known rotenone action is the inhibition of **mitochondrial complex I** blocking the ETC

It is also involved in promotion of **alpha-synuclein aggregation** and **microtubule destabilization**



► *Exp Neurobiol.* 2020 Jun 30;29(3):207–218. doi: [10.5607/en20014](https://doi.org/10.5607/en20014)

Death-associated Protein Kinase 1 Phosphorylates α-Synuclein at Ser129 and Exacerbates Rotenone-induced Toxic Aggregation of α-Synuclein in Dopaminergic SH-SY5Y Cells

► *Neurochem Res.* 2012 Oct;37(10):2178–89. doi: 10.1007/s11064-012-0841-y. Epub 2012 Jul 31.

Astrocyte activation: a key step in rotenone induced cytotoxicity and DNA damage

Supriya Swarnkar¹, Sarika Si
Chandishwar Nath

► *Recent Adv Inflamm Allergy Drug Discov.* 2022;15(1):35–43.
doi: 10.2174/2772270816666220105124730.

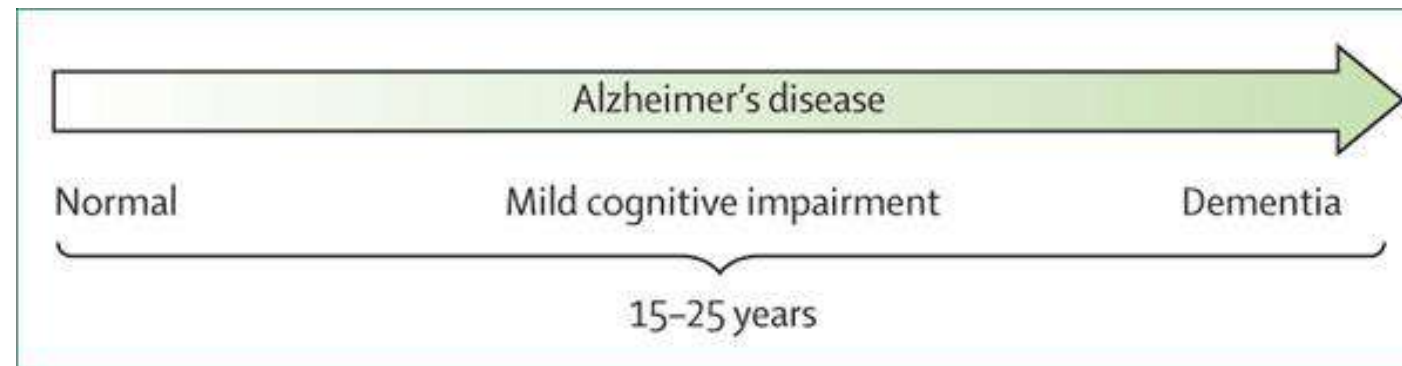
Neuroinflammation and Behavioral Deficit in Rotenone-Induced Neurotoxicity in Rats and the Possible Effects of Butanolic Extract of *Centaurea africana*

Sabrina Hadjira¹, Amira Mansour², Ramdane Seghiri², Ahmed Menad¹, Fadila Benayache²,
Samir Benayache², Squad Ameddah¹

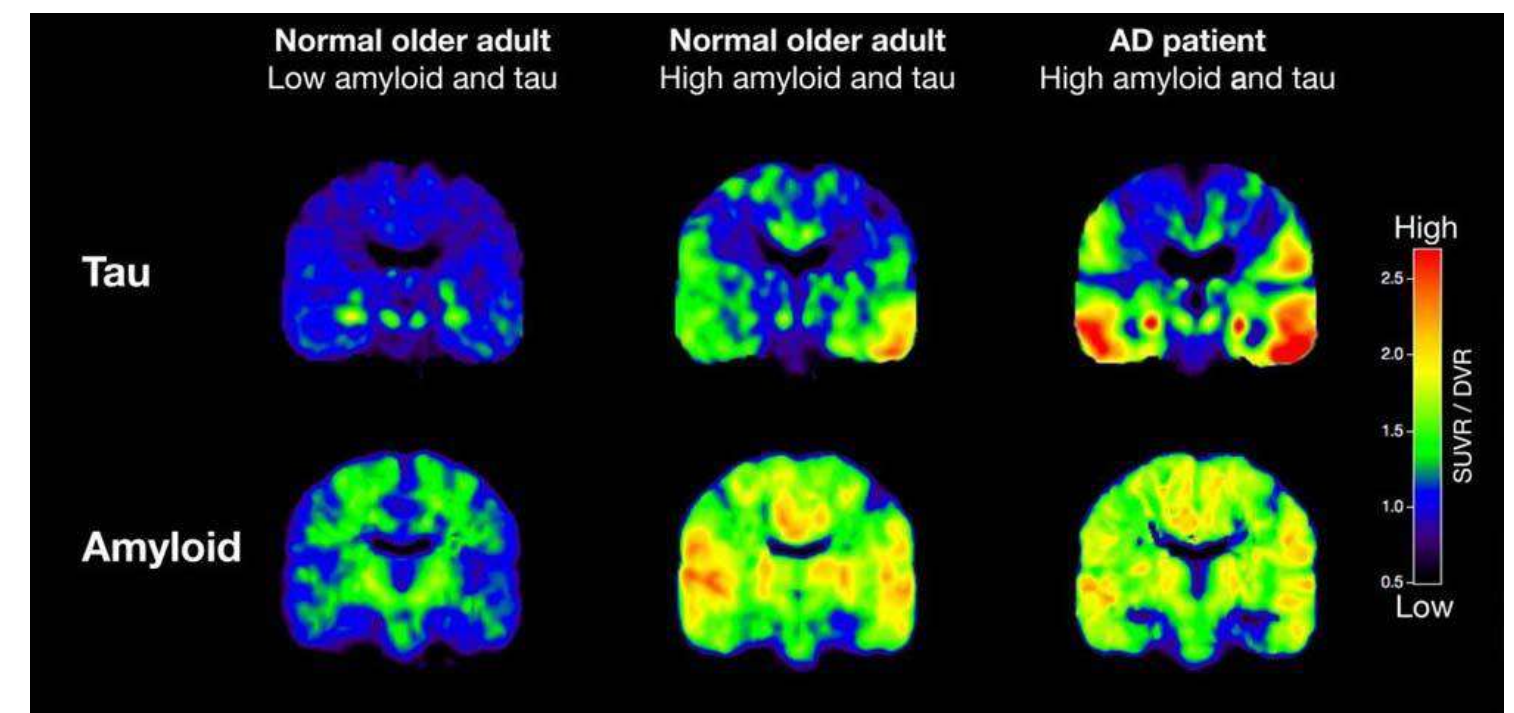
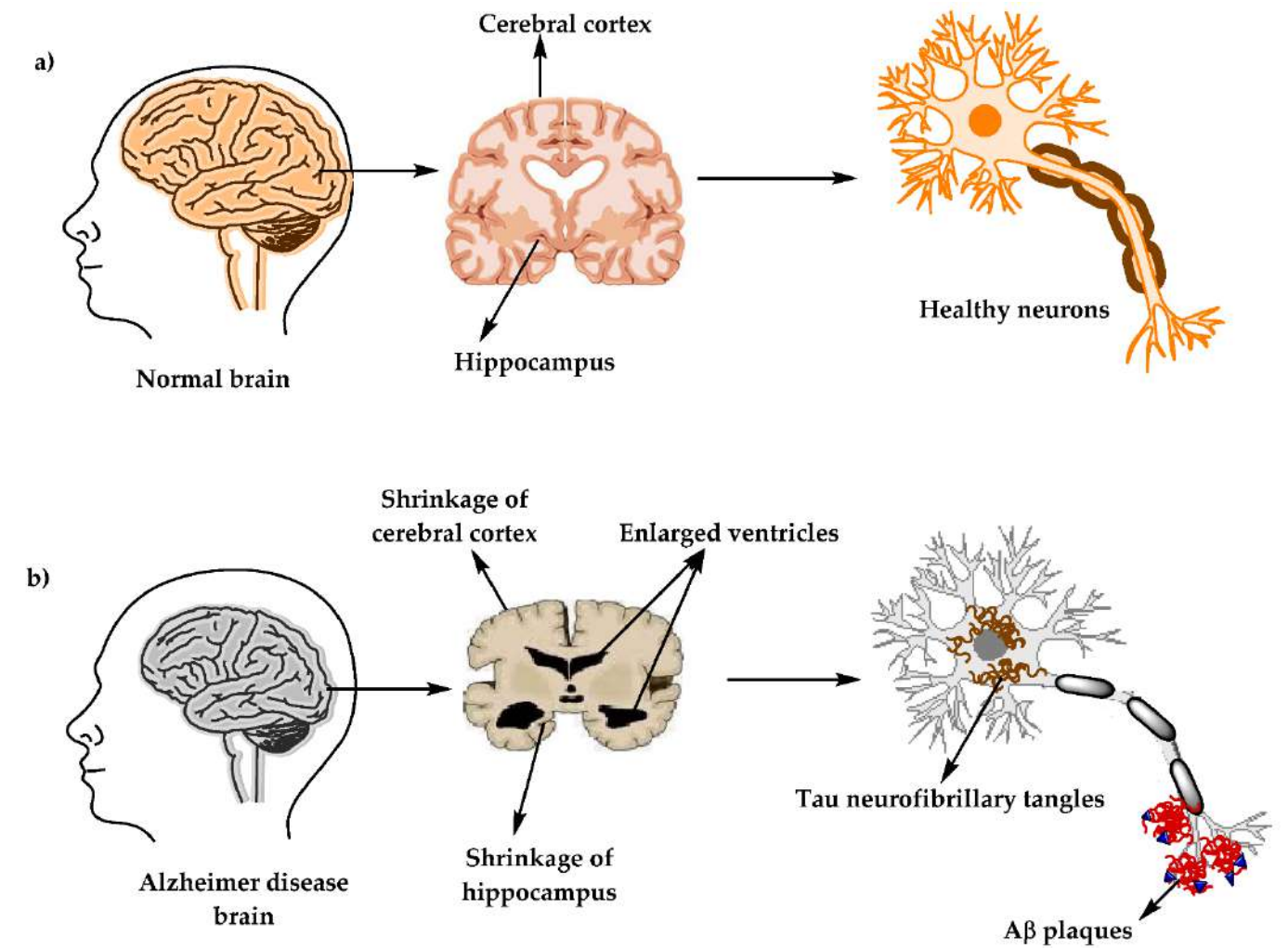
Rotenone triggers a neurotoxic circle where neurons, microglia and astrocytes interact, amplifying inflammation, oxidative stress and synaptic degeneration.

- Commonest form of dementia
- Characterized by the presence of **amyloid β (A β)** extracellular plaques and intracellular neurofibrillary tangles (NFT), composed of **hyperphosphorylated tau protein**
- In addition, synaptic loss, mitochondrial dysfunction, neuroinflammation, and disrupted calcium homeostasis have been observed in AD patients.

AD is a *continuum*



- Dementia is the end-point of a long-time presence of AD pathology.



Correlation between AD histopathological hallmarks and dementia has been challenged by the identification of “**Non-Demented with Alzheimer’s Neuropathology**” (NDAN) individuals.



cognitively intact despite displaying pathologic features of AD.

Some neuroprotective mechanisms prevent cognitive decline and preserve synaptic functional integrity.

► *Brain Pathol.* 2023 Jan;33(1):e13108. doi: 10.1111/bpa.13108. Epub 2022 Jul 11.

TREM2-induced activation of microglia contributes to synaptic integrity in cognitively intact aged individuals with Alzheimer's neuropathology

Anna Fracassi¹, Michela Marcatti¹, Batbayar Tumurbaatar¹, Randall Woltjer², Sandra Moreno³, Giulio Taglialetela¹

► *J Neurosci.* 2021 Jan 20;41(3):538–554. doi: [10.1523/JNEUROSCI.0295-20.2020](https://doi.org/10.1523/JNEUROSCI.0295-20.2020)

Oxidative Damage and Antioxidant Response in Frontal Cortex of Demented and Nondemented Individuals with Alzheimer's Neuropathology

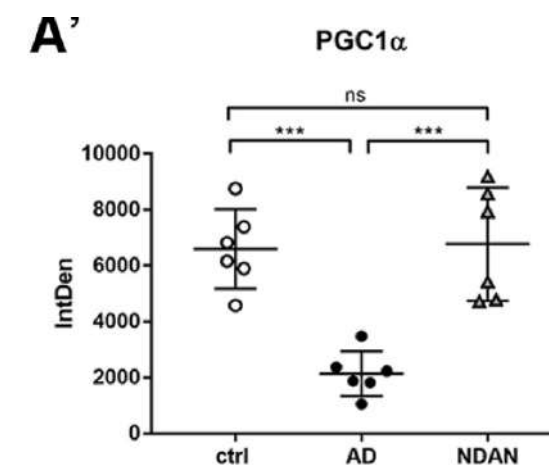
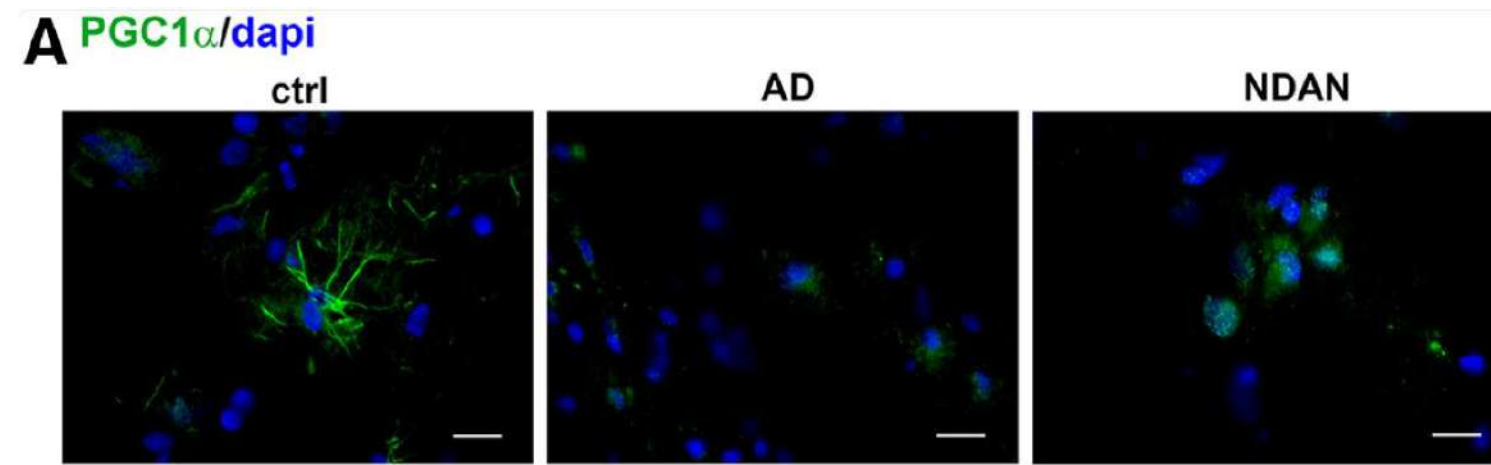
Anna Fracassi¹, Michela Marcatti¹, Olga Zolochovska¹, Natalie Tabor², Randall Woltjer³, Sandra Moreno^{4,✉}, Giulio Taglialetela^{1,✉}

► *Alzheimers Dement.* Author manuscript; available in PMC: 2024 Dec 1.

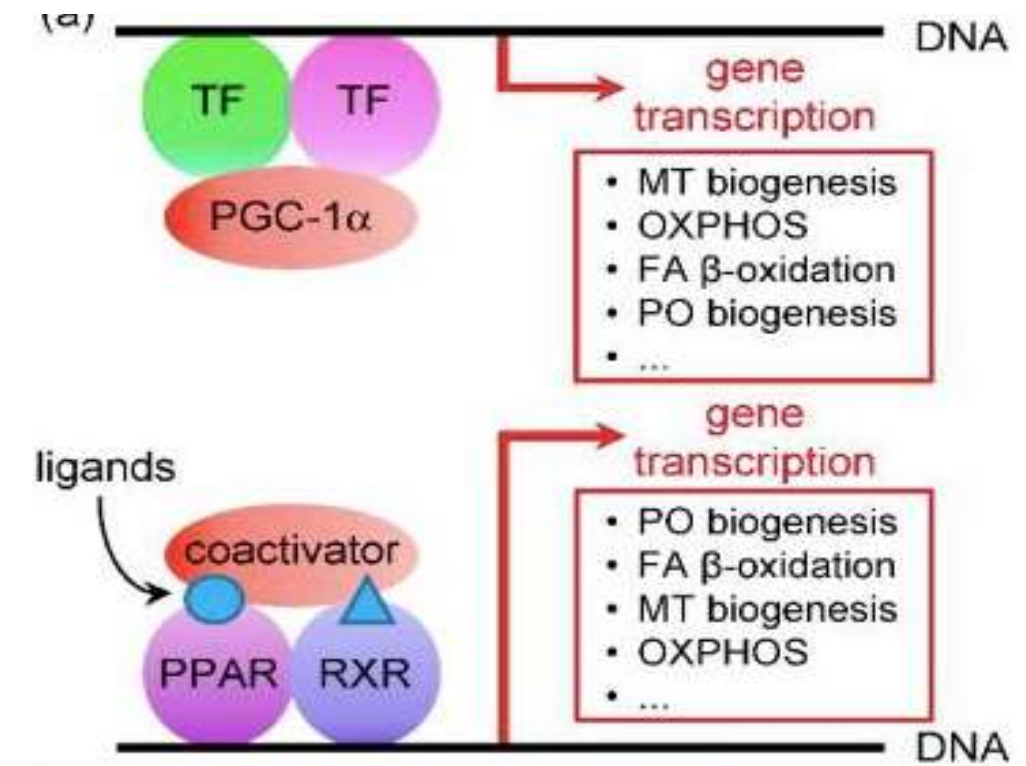
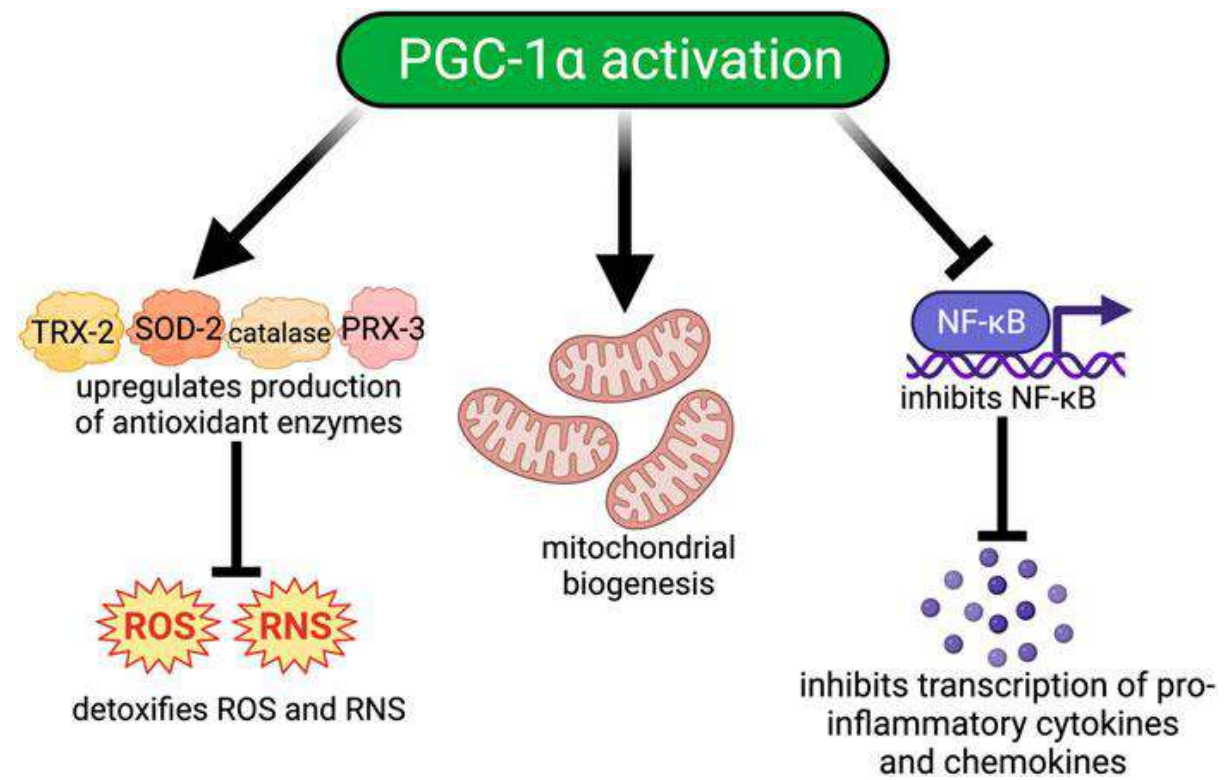
Published in final edited form as: *Alzheimers Dement.* 2023 May 16;19(12):5355–5370. doi: [10.1002/alz.13074](https://doi.org/10.1002/alz.13074)

Preserved Autophagy in Cognitive Intact Non-Demented Individuals with Alzheimer’s Neuropathology

Batbayar Tumurbaatar^{1,✉}, Anna Fracassi^{1,✉}, Pietro Scaduto^{1,✉}, Jutatip Guptarak¹, Randall Woltjer², Daniel Jupiter³, Giulio Taglialetela¹

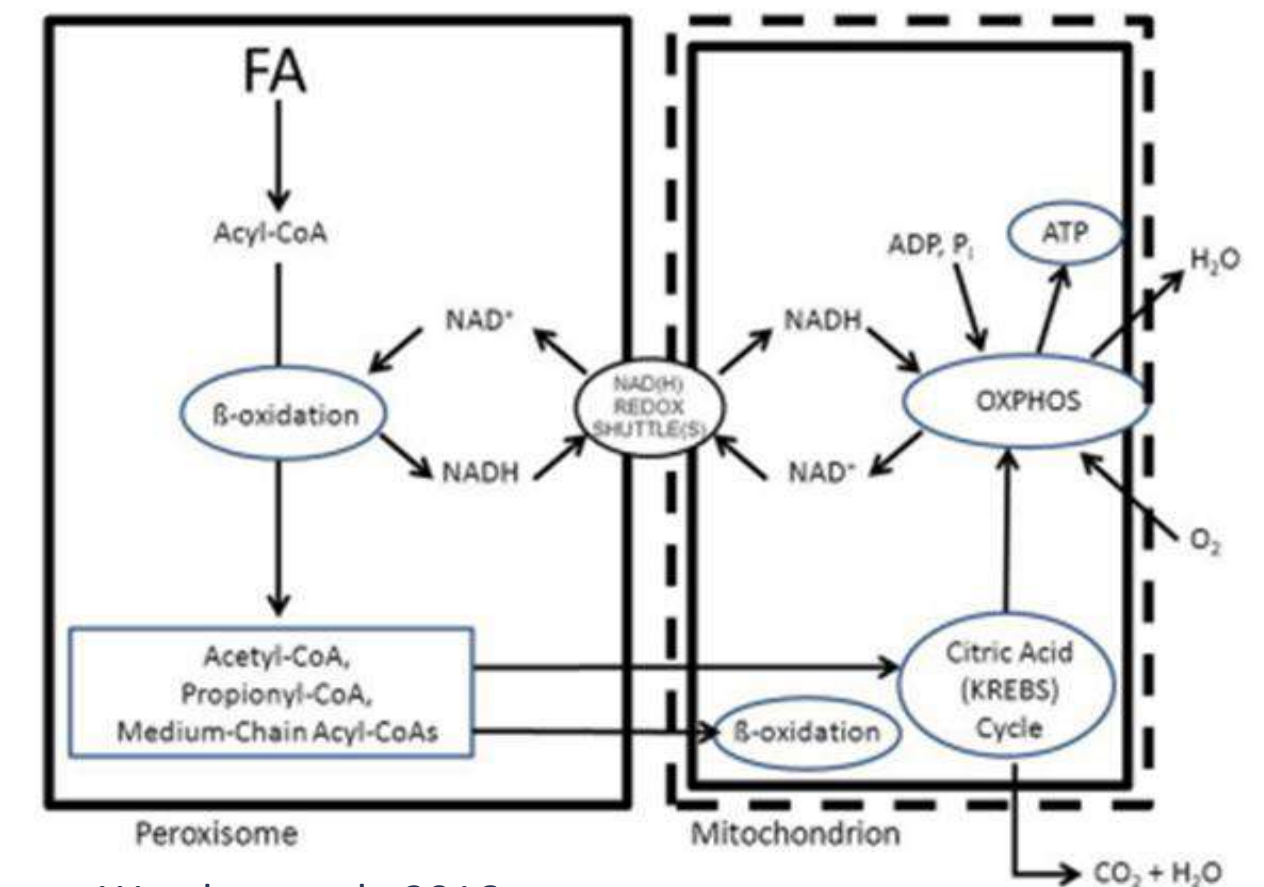
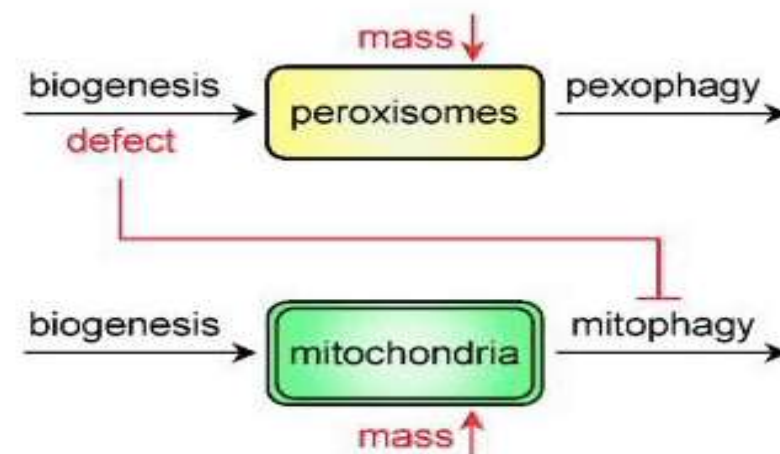


NDAN individuals display PGC1 α expression levels similar to those of age-matched controls while lower levels are found in AD individuals.



Modificato da Fransen et al. 2017.

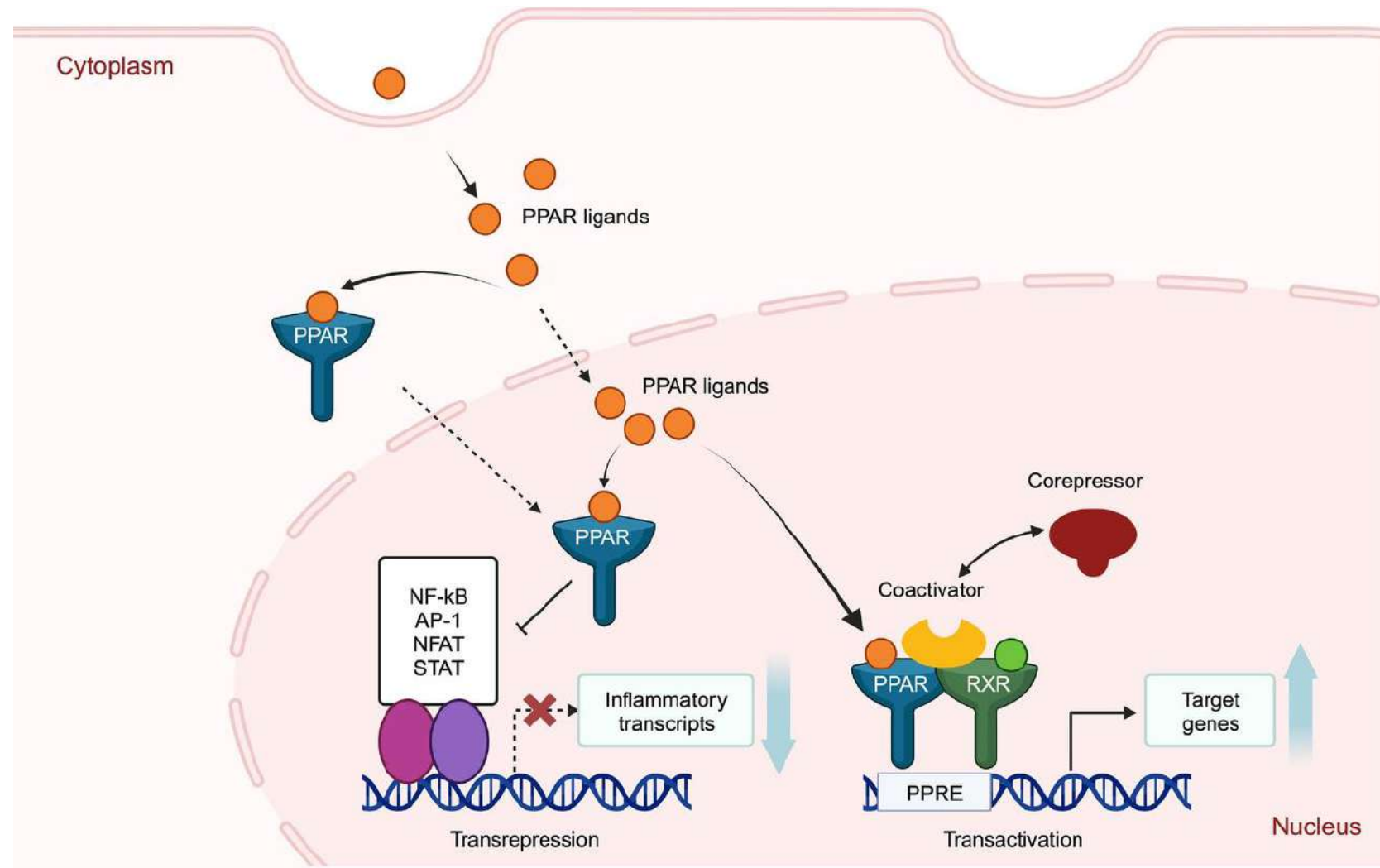
The sharing of common transcription factors demonstrates the strong relationship between mitochondria and peroxisomes, which implies the interdependence of metabolic and biogenetic pathways.



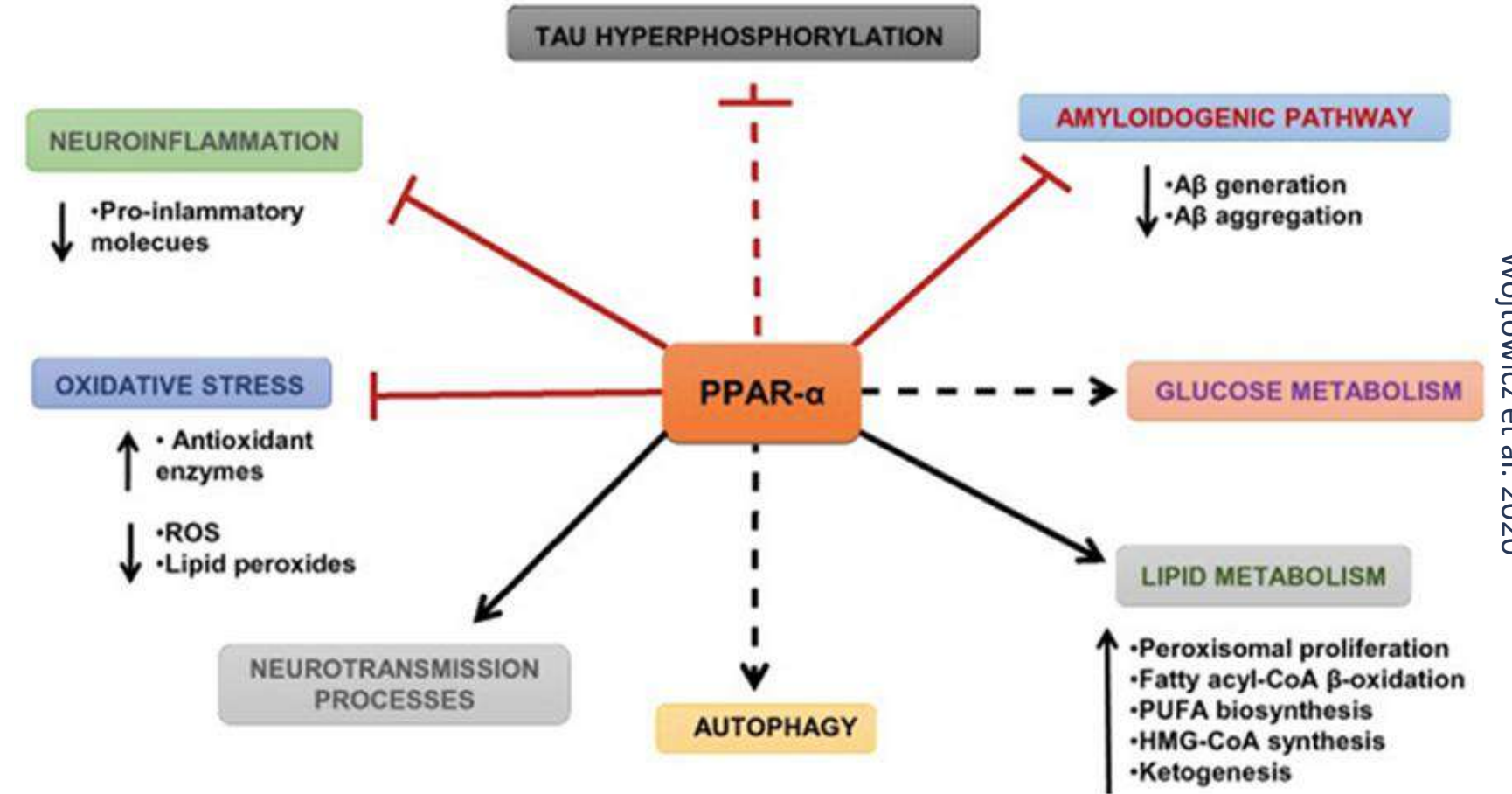
Wanders et al., 2016

Modificato da Fransen et al. 2017.

- PPAR- α heterodimerizes with retinoid X receptor (RXR)
- binds to response elements (PPRE) in the promoter region of target genes
- acts as potent transcription factor



Trends in Pharmacological Sciences



Wójtowicz et al. 2020

PPAR- α agonist can be recognized as a potential therapeutic tool for neurodegenerative diseases.

[Hum Mol Genet.](#) 2016 Jan 15; 25(2): 317–327.

PMCID: PMC4706116

Published online 2015 Nov 24. doi: [10.1093/hmg/ddv477](https://doi.org/10.1093/hmg/ddv477)

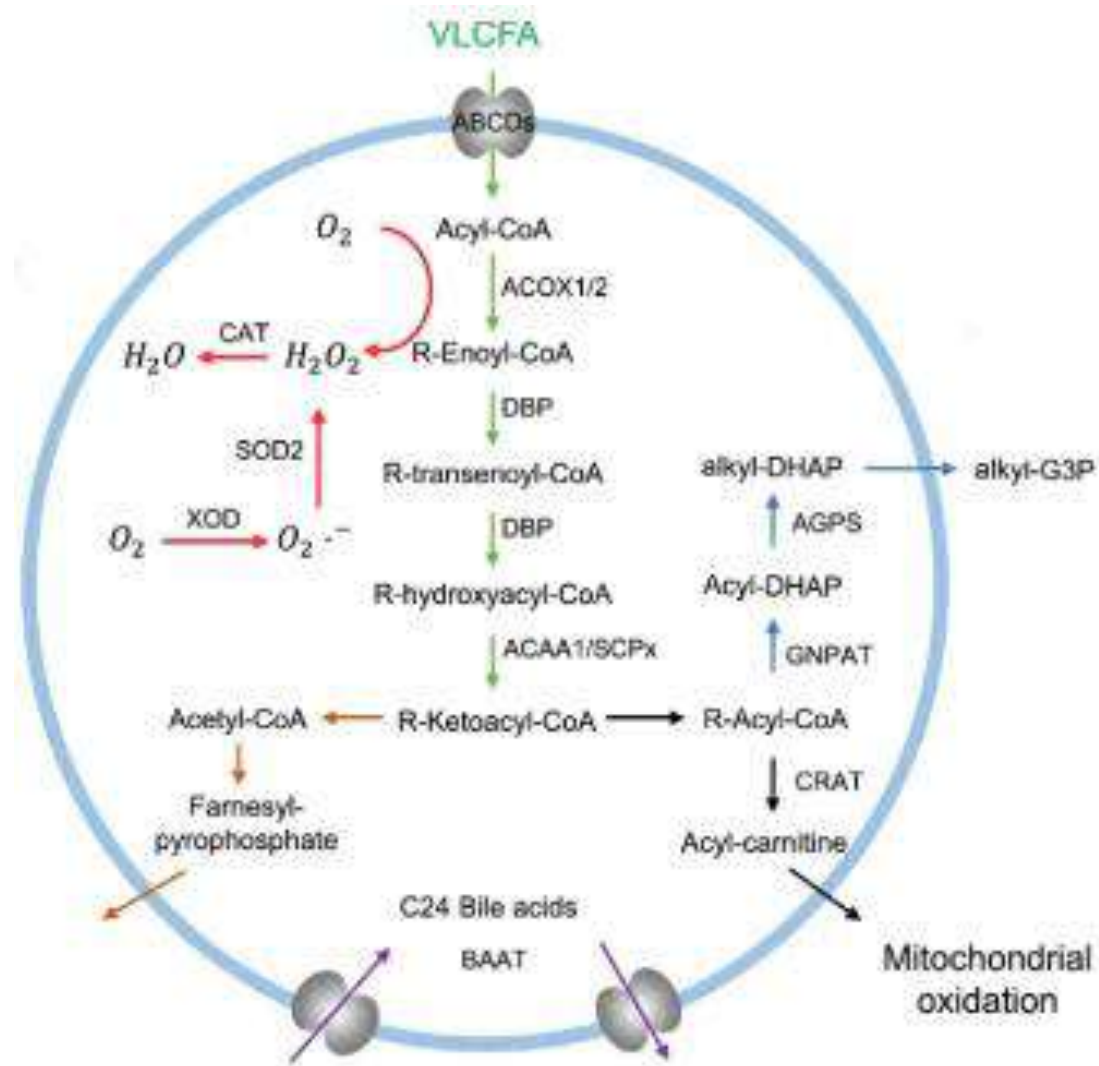
PMID: [26604138](https://pubmed.ncbi.nlm.nih.gov/26604138/)

Preferential PPAR- α activation reduces neuroinflammation, and blocks neurodegeneration *in vivo*

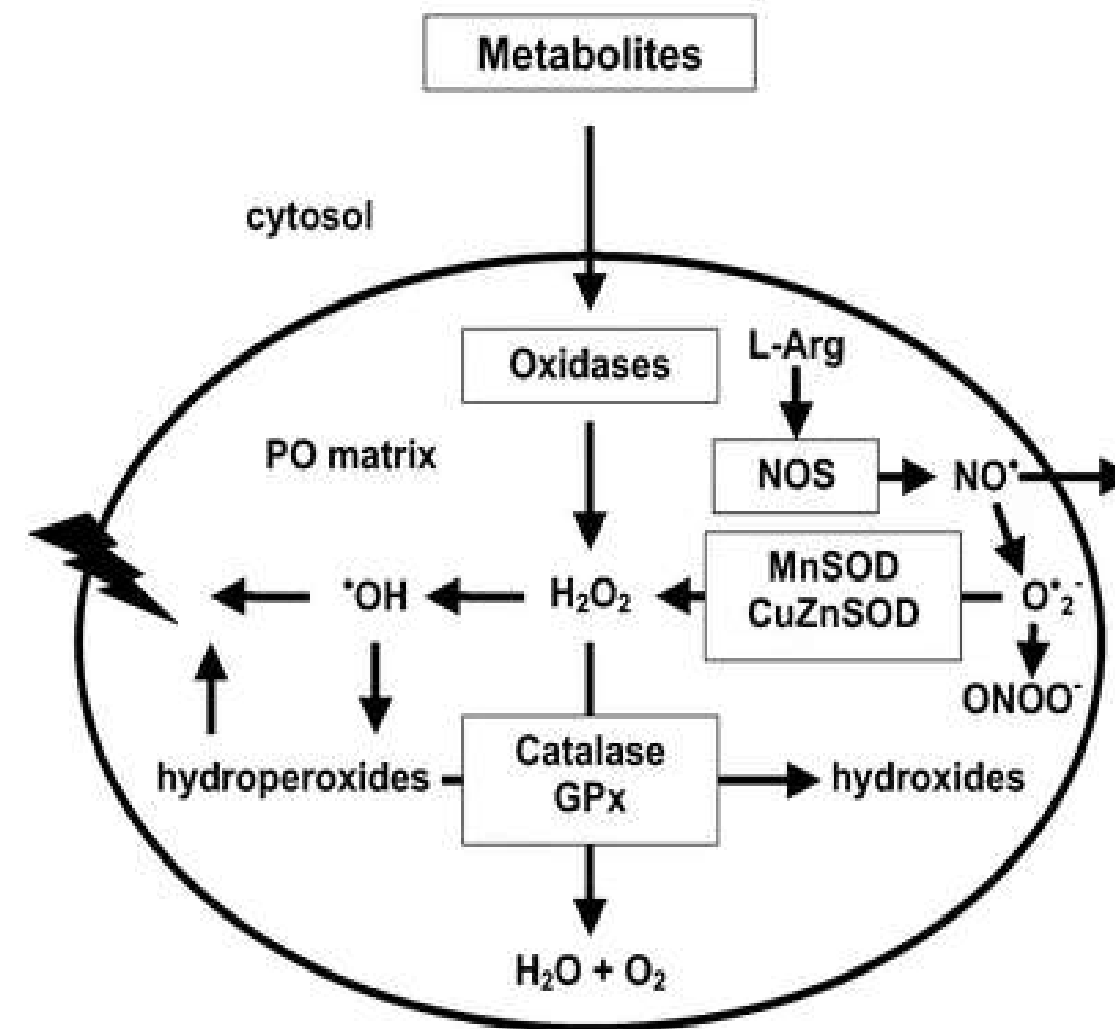
[Mohammad A. Esmaili](#)^{1,††}, [Shilpi Yadav](#)^{1,†}, [Ravi Kr. Gupta](#)², [Garrett R. Waggoner](#)¹, [Abigail Deloach](#)¹, [Noel Y. Calingasan](#)³, [M. Flint Beal](#)³, and [Mahmoud Kiaei](#)^{1,*}

PEROXISOMES

- FA β -OXIDATION



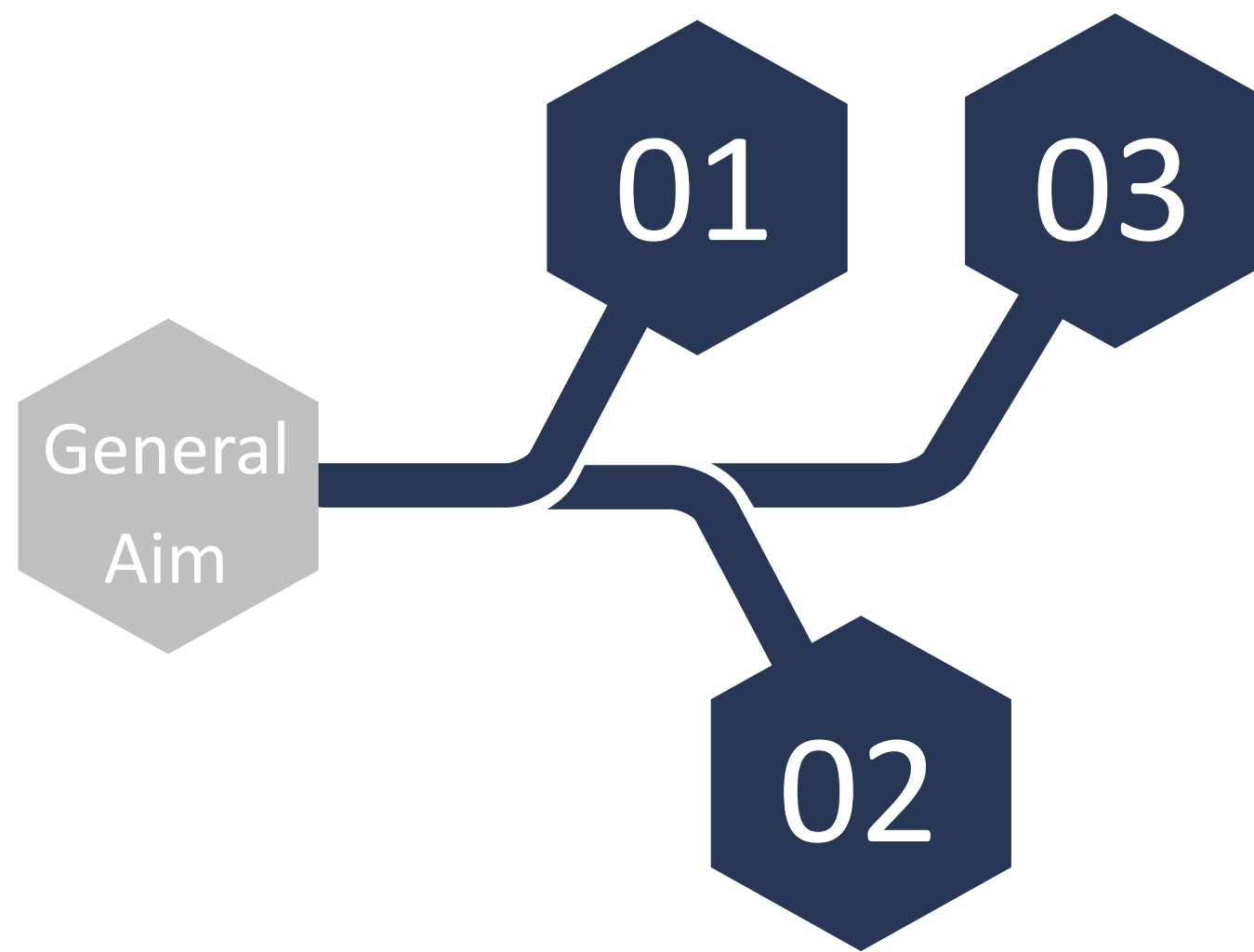
- ROS METABOLISM



General Aim:

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This integrated approach will provide a more comprehensive and detailed view of the structural, ultrastructural, and functional changes occurring in neural cells during neurodegeneration.



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PHASE-1

PHASE-1

PHASE-1



Roma Tre



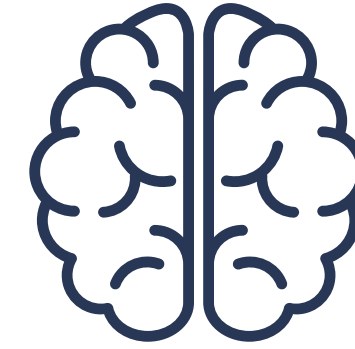
Investigating the role of peroxisomes in a cellular model of PD neurodegeneration and evaluating the effect of neuroprotective agents:

- Setting BV-2 cell cultures treated with rotenone to induce PD-like phenotype.
- WB/IF: markers of microglial activation and peroxisomes (IBA1, PMP70, ACOX1, ACAA1, CAT, SOD1, SOD2, PPAR α and PGC-1 α)
- ELISA: proinflammatory cytokines (TNF α , IL-6, etc.)
- Treatments with PPAR α agonists

PHASE-2

PHASE-2

PHASE-2



Characterizing the involvement of peroxisomes in NDAN human neocortex vs. AD individuals:

- WB/IF/IHC: peroxisomal markers and selective autophagy
- TEM: ultrastructure of peroxisomes and mitochondria

Extraction and purification of pTau from NDAN and AD individuals:

- Immunoprecipitation with pTau-specific antibodies
- Characterization of NDAN pTau oligomers

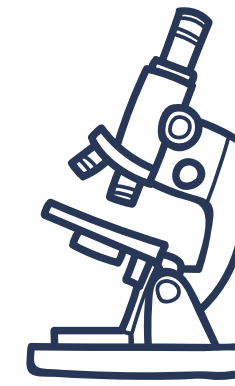
PHASE-3

PHASE-3

PHASE-3



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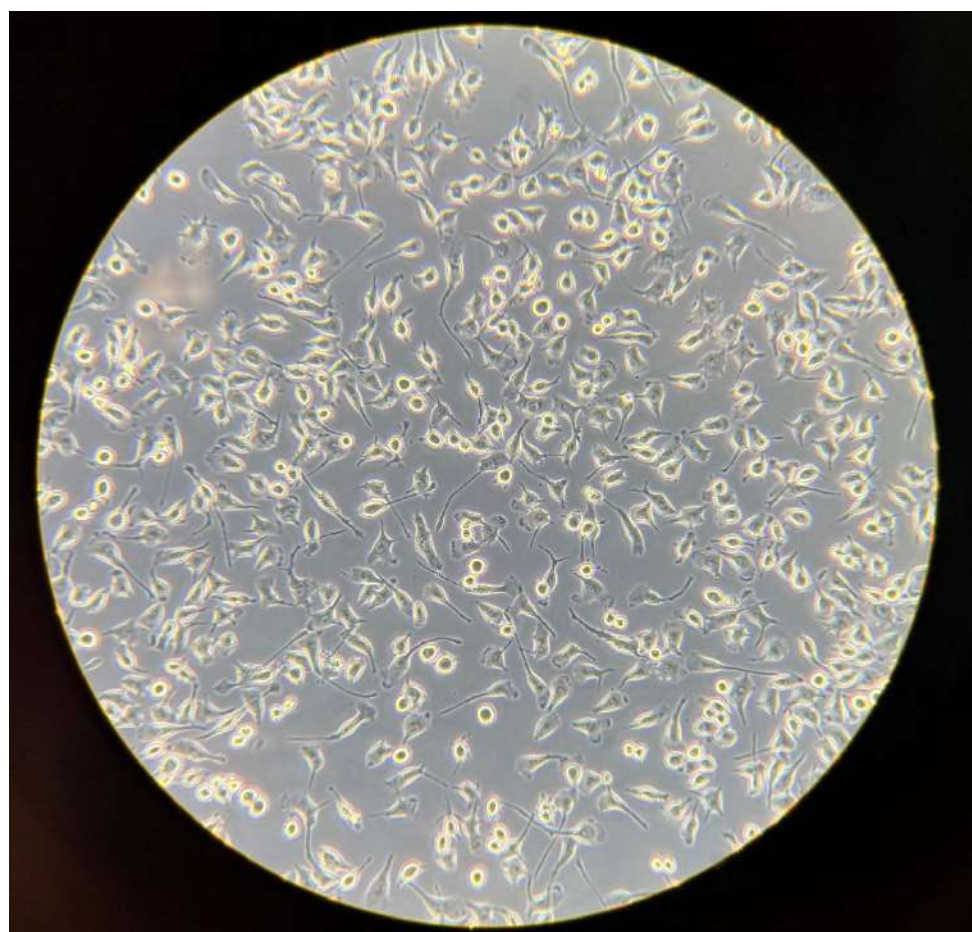


Testing the effects of NDAN pTau administration with or without neuroprotective agents:

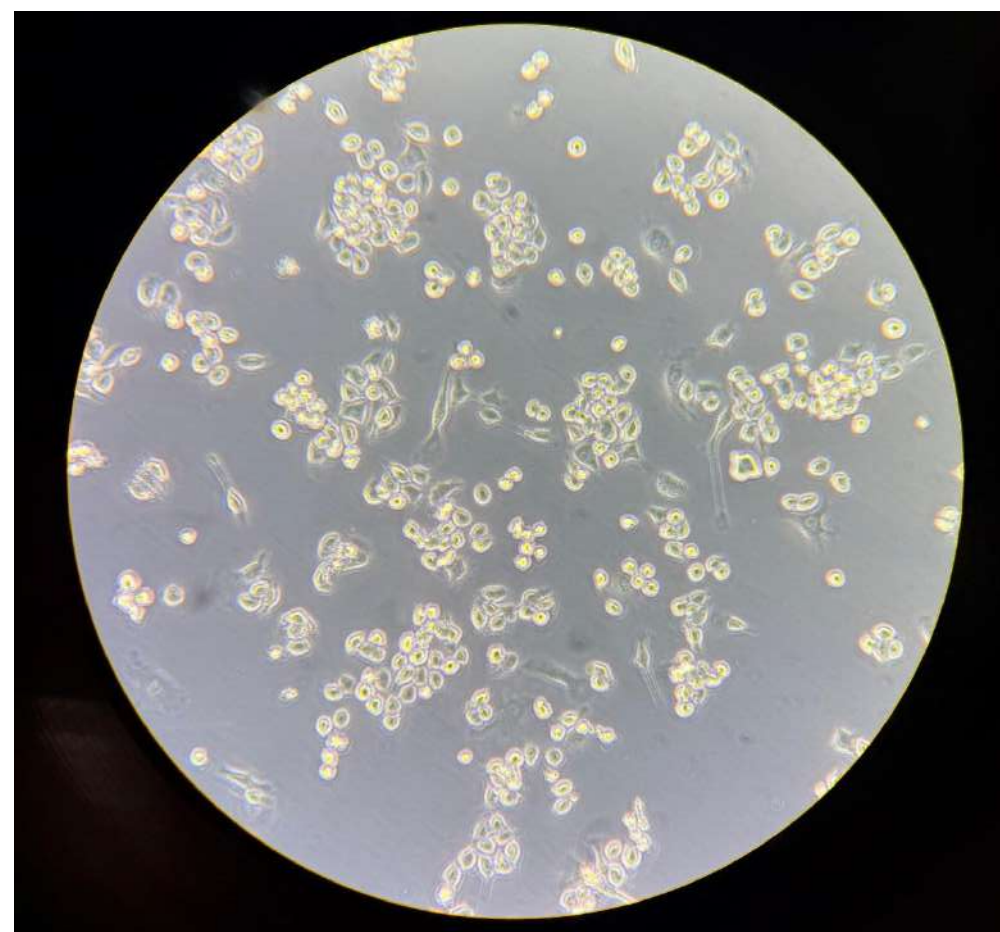
- Treatment of differentiated SH-SY5Y and BV-2 cells with pTau
- WB/IF/IHC: Evaluating antioxidant response, mitochondrial and peroxisomal functions
- FIB/SEM: mitochondrial, peroxisomal morphology and selective autophagy
- Treatments with PPAR α agonists

*Thank you
for your attention!*

CTRL



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