

Air pollution-derived ultrafine particles induce neurological disorders in mice and differentiated human dopaminergic neuronal cells

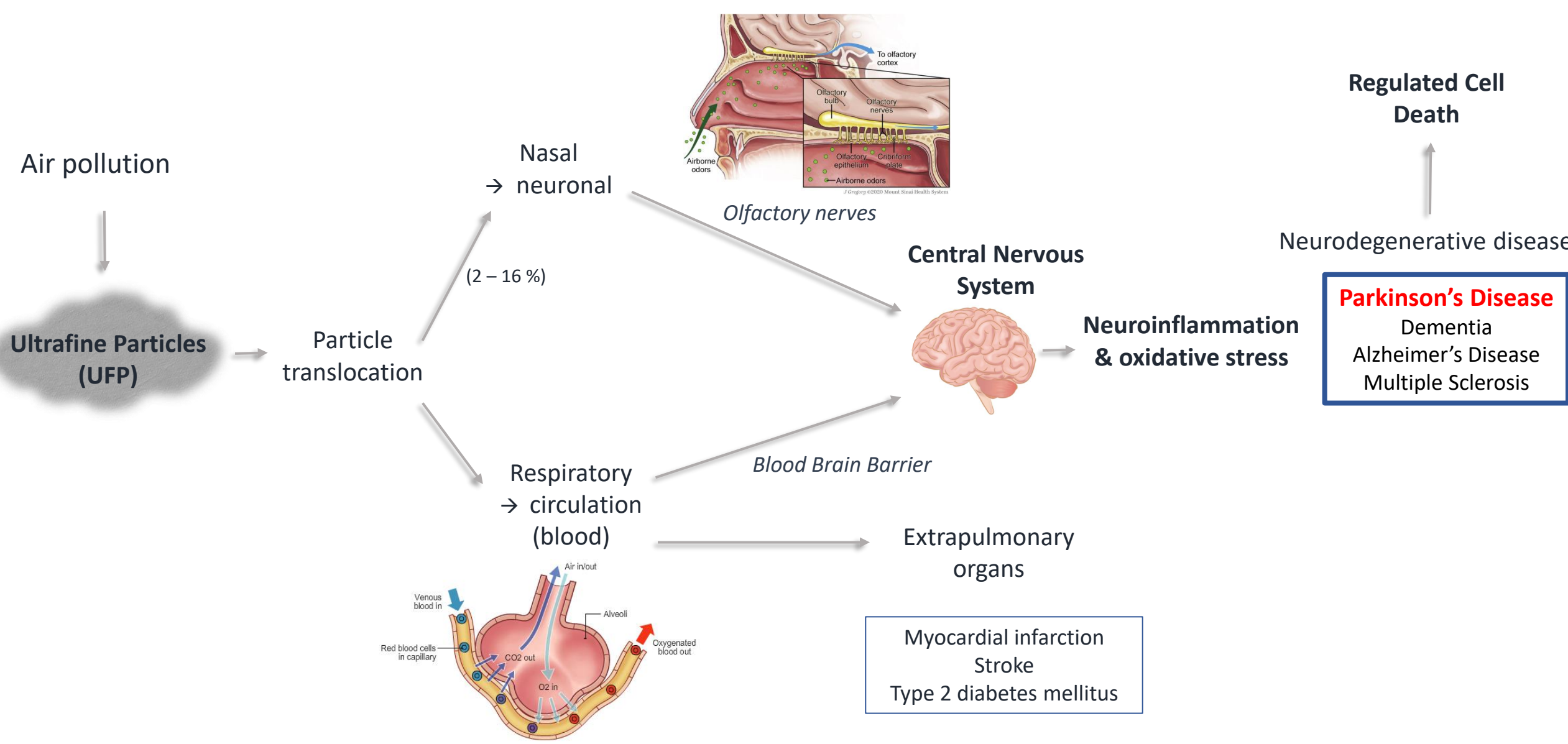
Emma Theerens, J. Carpentier, E. Barbier, K. Vanbrabant, O. Simonin, K. Timmerman, C. Laloux, A. Jonneaux, J.-M. Lo Guidice, D. Devos, G. Garçon

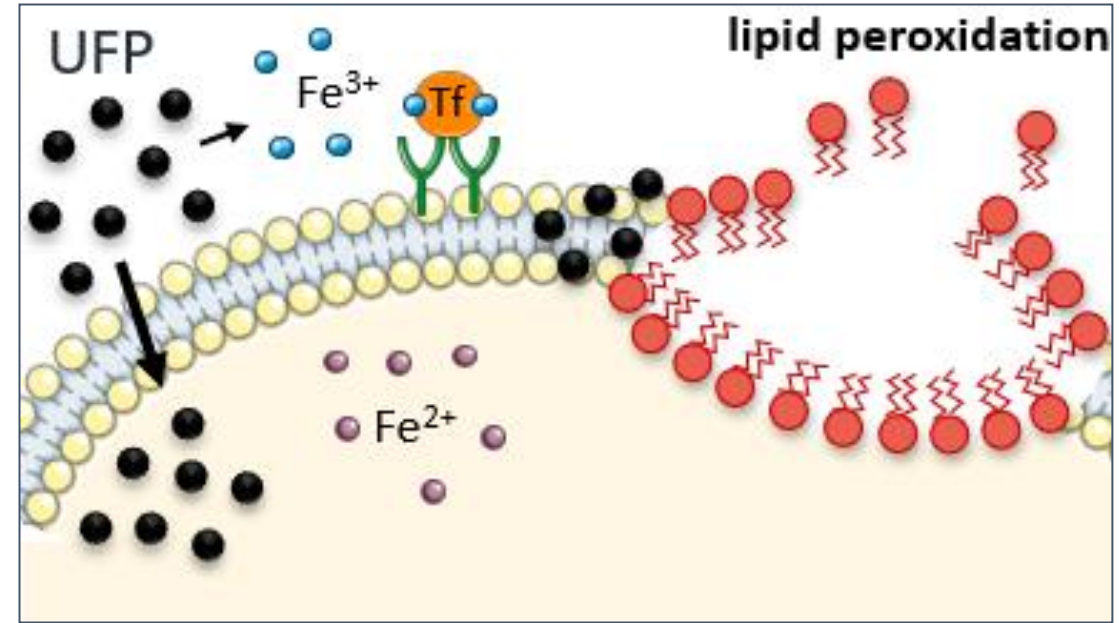
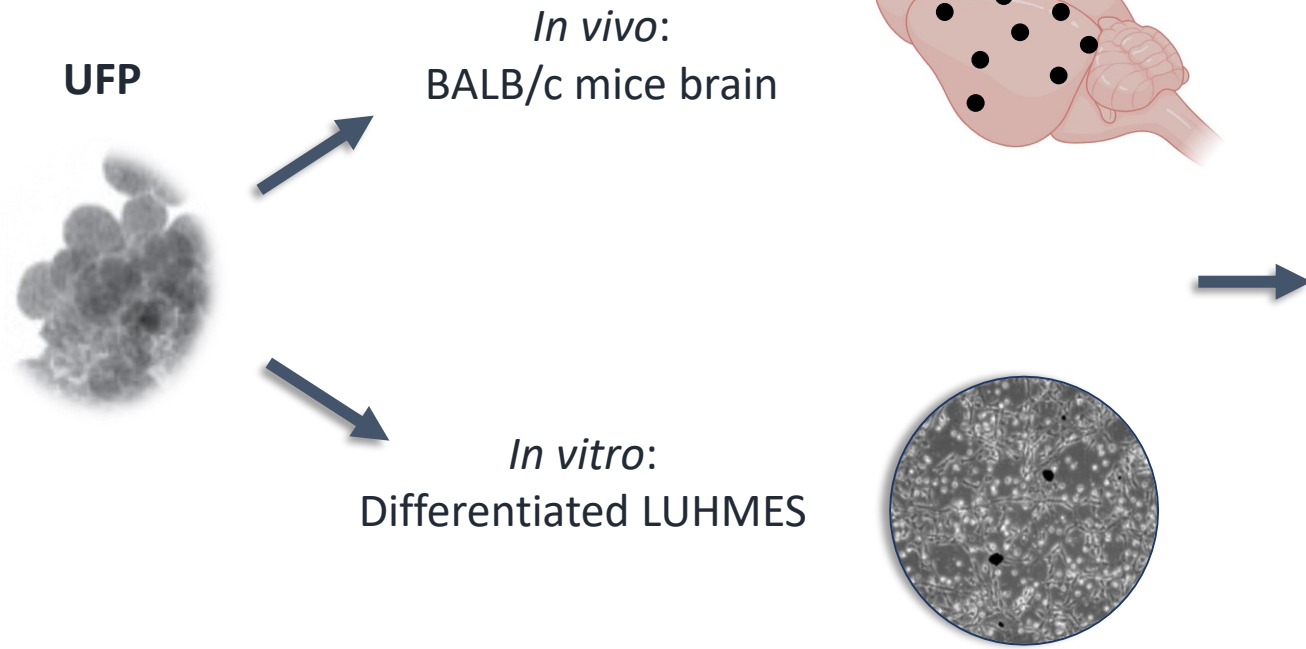


4/07/2024 (Brussels)

Ultrafine Particles – Air Quality and Climate







oxidative stress / lipid peroxidation

regulated cell death
(apoptosis, autophagy, **ferroptosis**)

UFP



- Capital of Hauts-de-France
- ± 35 km²
- 1.1 million inhabitants (metropolitan)

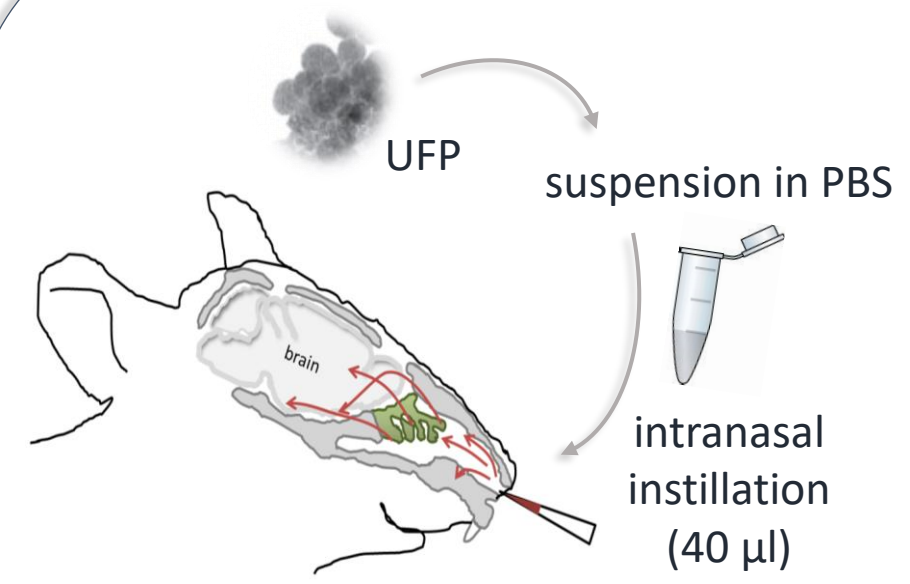


Dekatti DGI-1570

Summer 2019

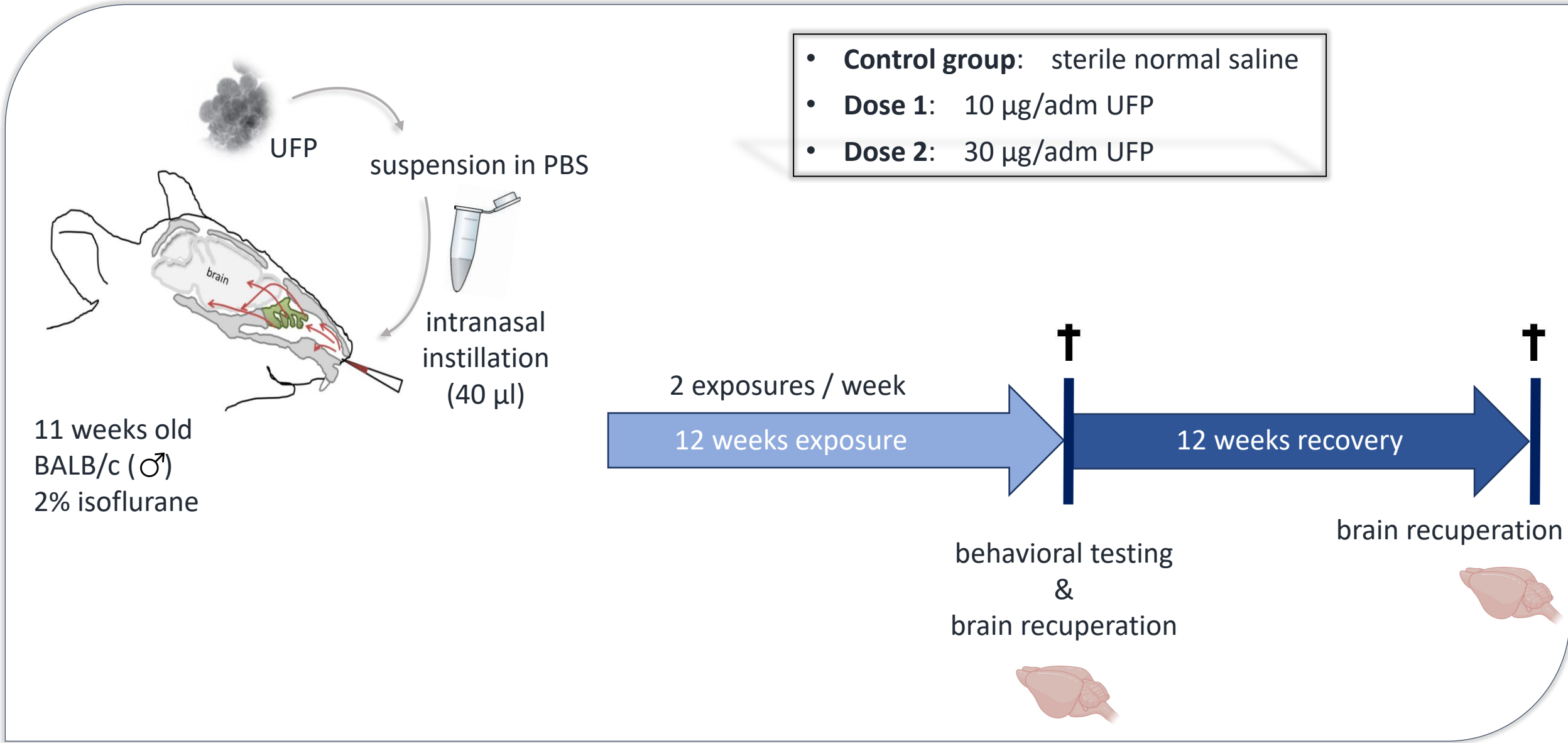
chemical composition:
e.g., Fe: 4.1 mg/g

EXPOSURE



11 weeks old
BALB/c (♂)
2% isoflurane

EXPOSURE



- **Control group:** sterile normal saline
- **Dose 1:** 10 µg/adm UFP
- **Dose 2:** 30 µg/adm UFP

11 weeks old
BALB/c (♂)
2% isoflurane

2 exposures / week
12 weeks exposure

12 weeks recovery

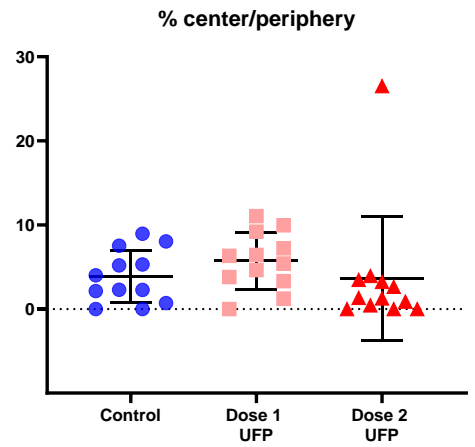
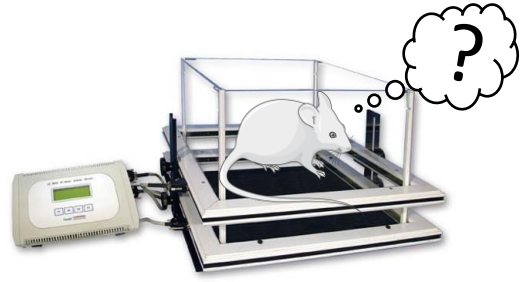
behavioral testing
&
brain recuperation

brain recuperation

Behavioural tests after subchronic exposure of UFP

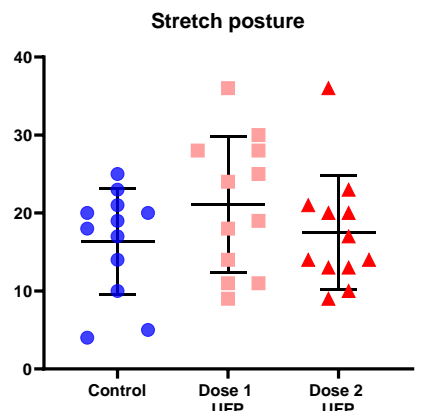
1 Motor function

Open field test



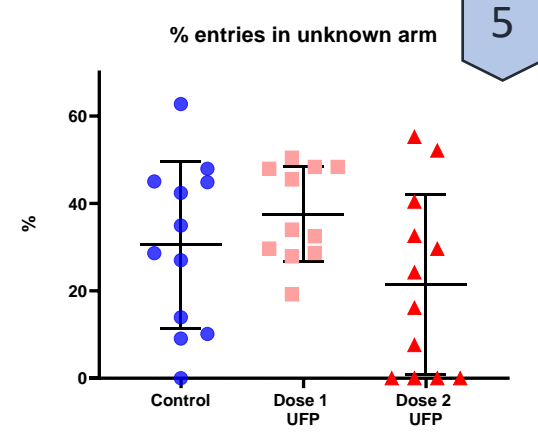
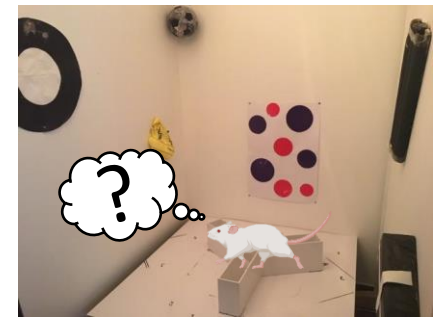
2 Anxiety

Elevated plus-maze

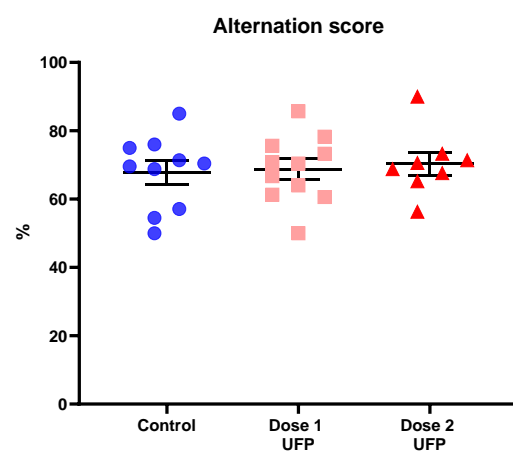
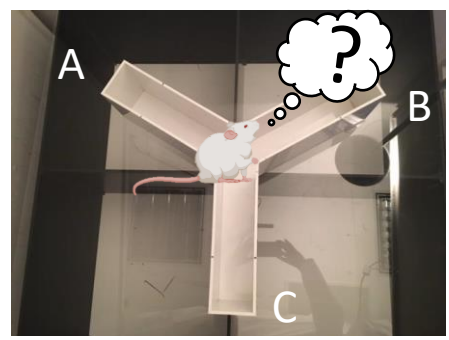


3 Cognition

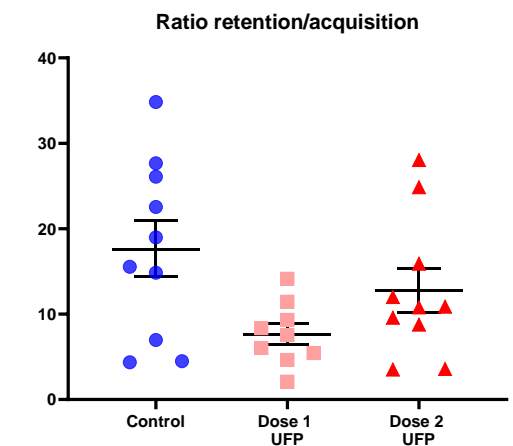
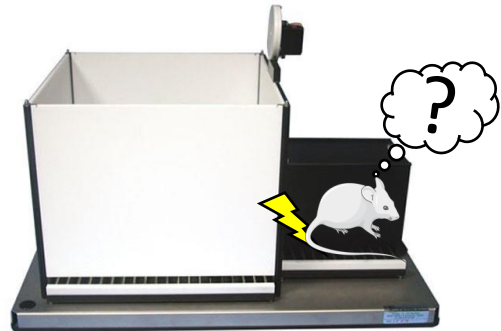
Y-maze



Spontaneous alternation

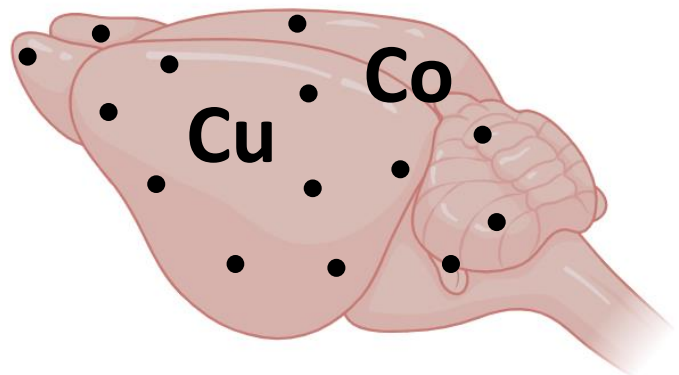


Passive avoidance test

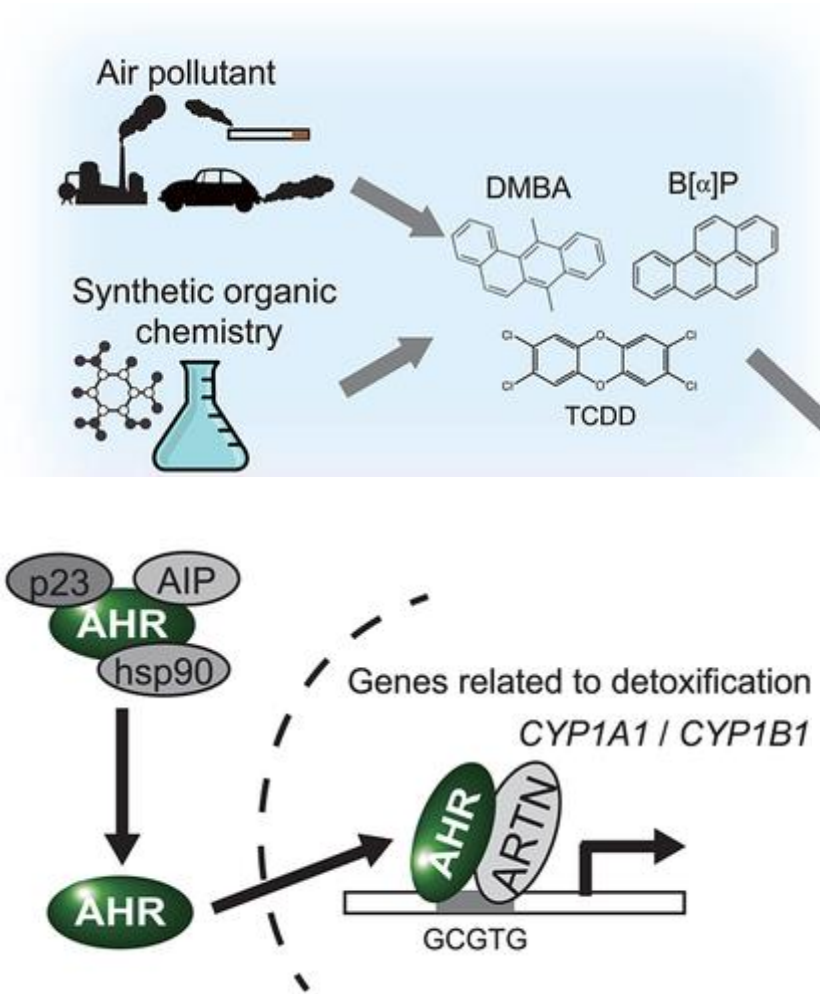


n = 12
 Non-parametric Mann-Whitney U test
 (GraphPad Prism8)
 Statistical analysis: * p<0.05; ** p<0.01.

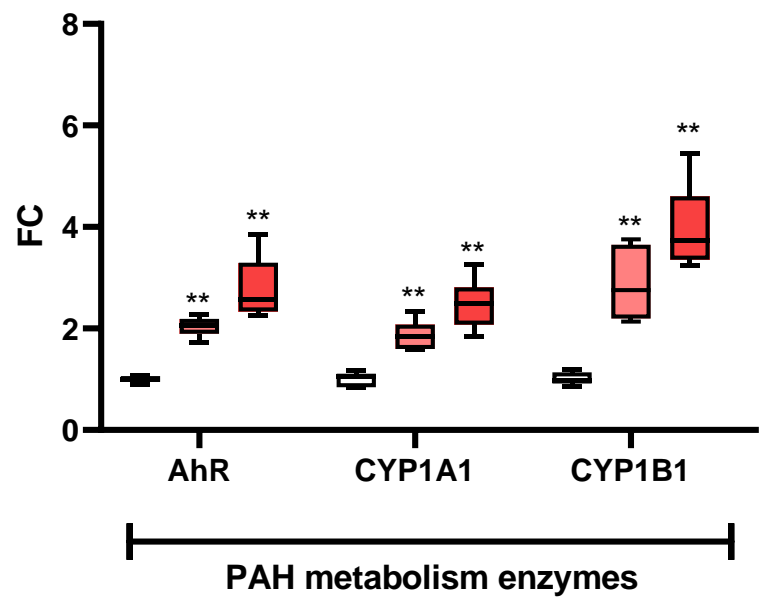
Metal tissue measurement by ICP-MS



Indirect UFP brain measurement



RT-qPCR gene expression of Polycyclic Aromatic Hydrocarbons (PAH) and metal metabolism enzymes

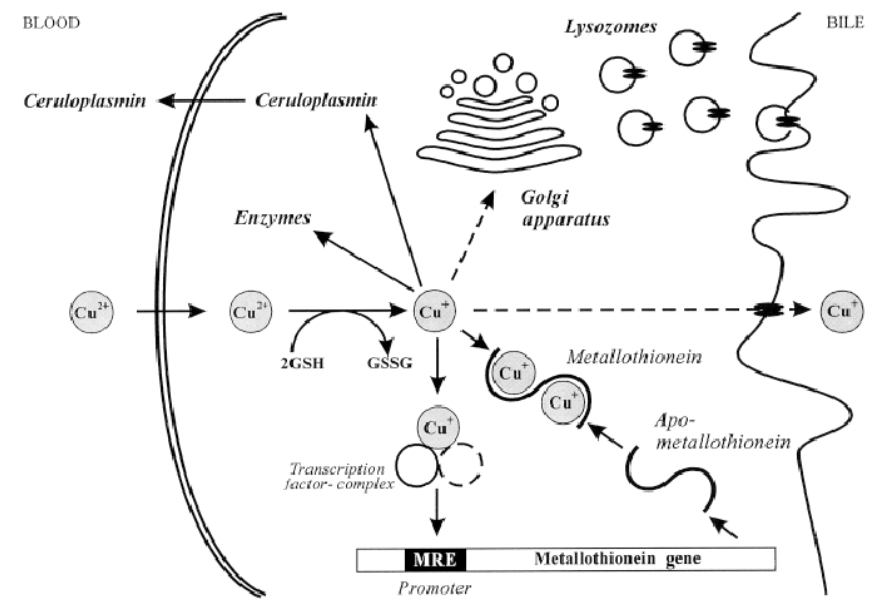


- Control (physiological serum)
- Dose 1 UFP (10 µg/adm)
- Dose 2 UFP (30 µg/adm)

(Adapted figure of Hidakay et al., 2019)

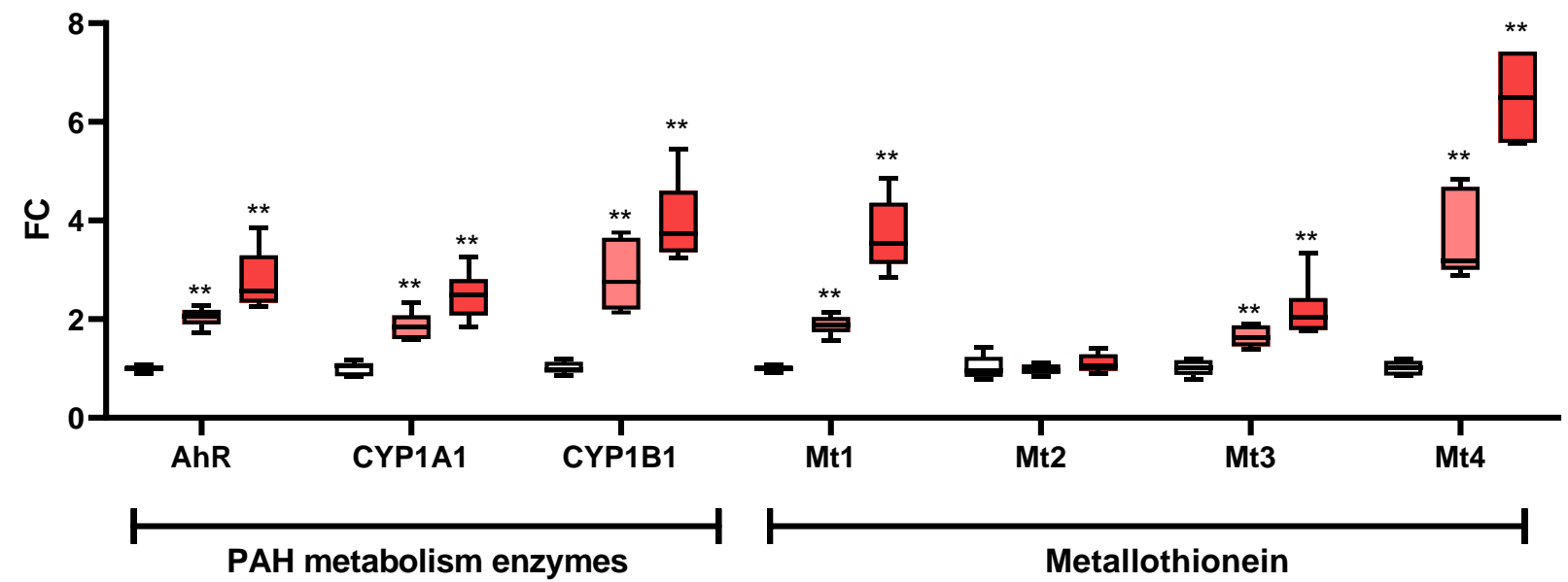
n = 6
 Non-parametric Mann-Whitney U test
 (GraphPad Prism8)
 Statistical analysis: * p<0.05; ** p<0.01.

Indirect UFP brain measurement



(Figure of Dameron et al., 1998)

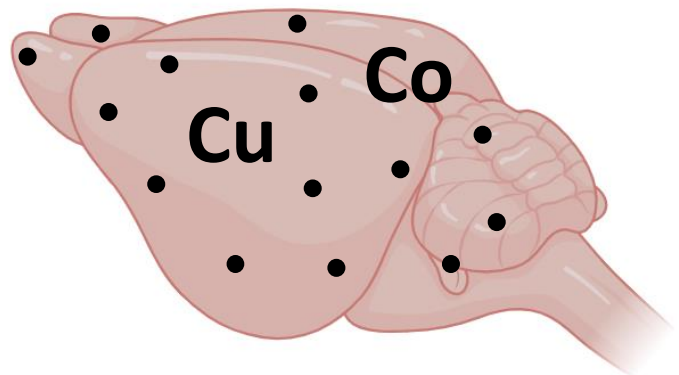
RT-qPCR gene expression of Polycyclic Aromatic Hydrocarbons (PAH) and metal metabolism enzymes



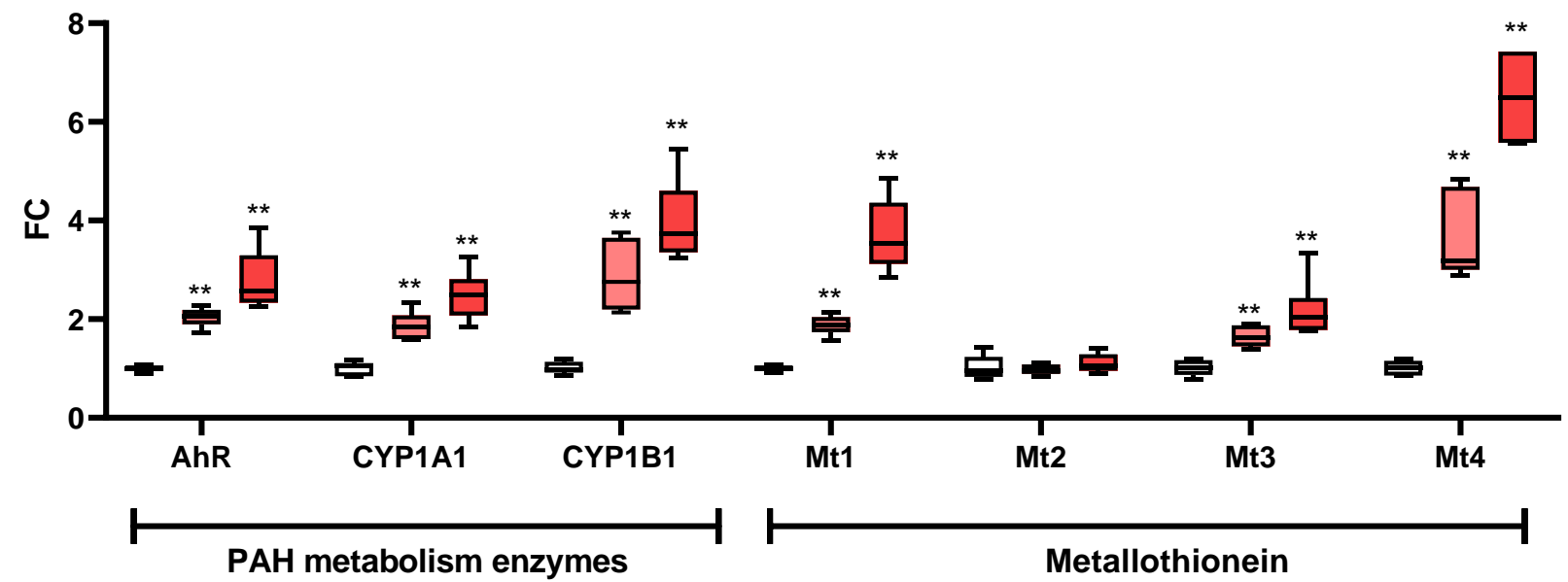
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n = 6
 Non-parametric Mann-Whitney U test
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 Statistical analysis: * p < 0.05; ** p < 0.01.

Metal tissue measurement by ICP-MS



RT-qPCR gene expression of Polycyclic Aromatic Hydrocarbons (PAH) and metal metabolism enzymes

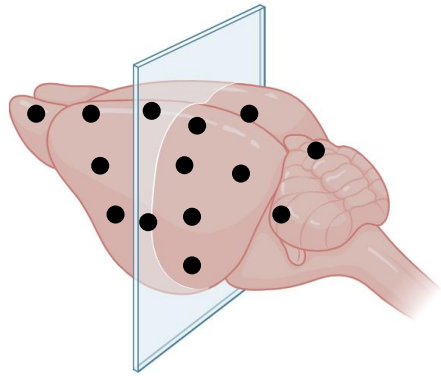


- Control (physiological serum)
- Dose 1 UFP (10 µg/adm)
- Dose 2 UFP (30 µg/adm)



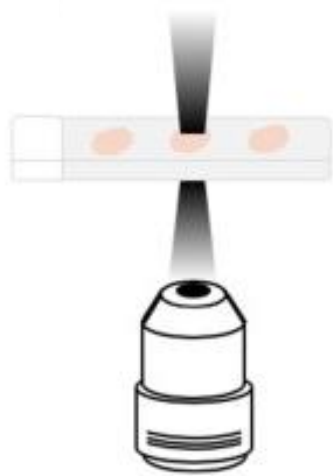
Direct UFP brain measurement

- Control (sterile saline/I.N.)
- UFP (30 µg/I.N.)



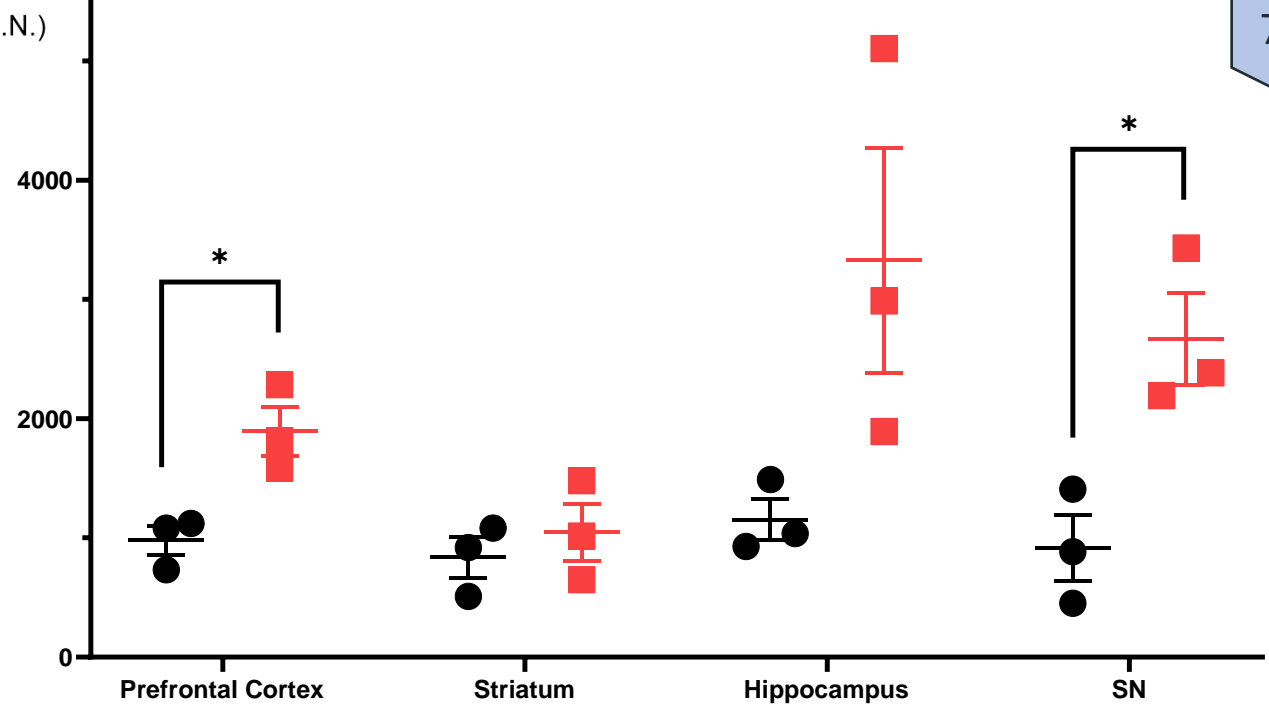
coronal sections
4 brain regions

3 months of
UFP exposure



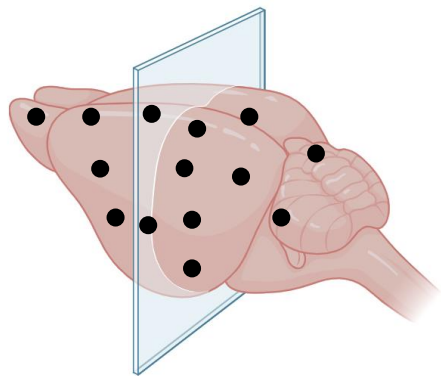
Femtosecond pulsed laser illumination
White light emission

no. of UFP/mm³ brain tissue



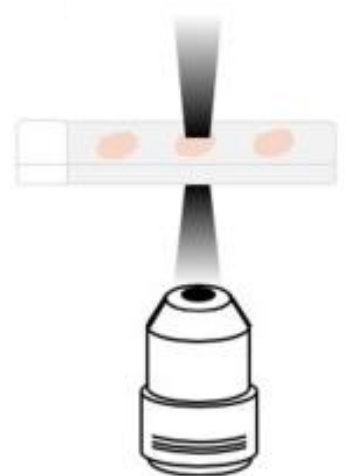
Direct UFP brain measurement

● Control (sterile saline/I.N.)
■ UFP (30 µg/I.N.)



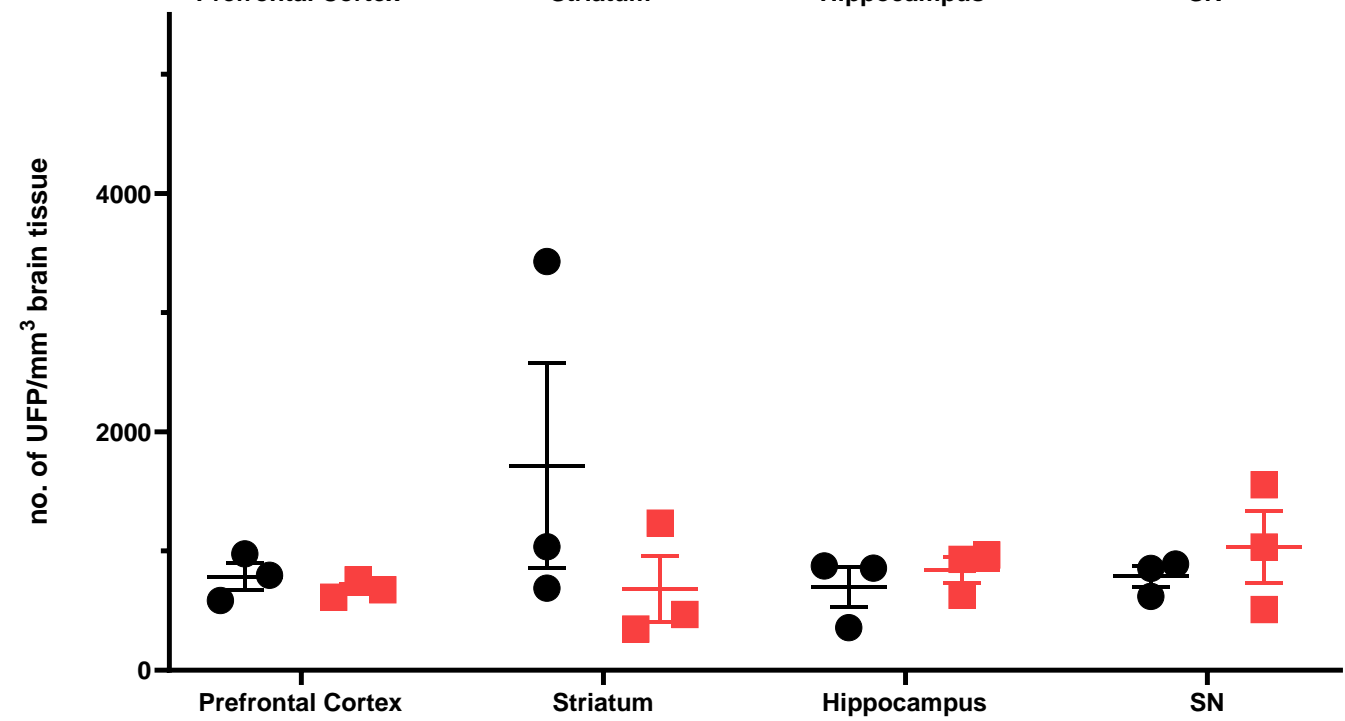
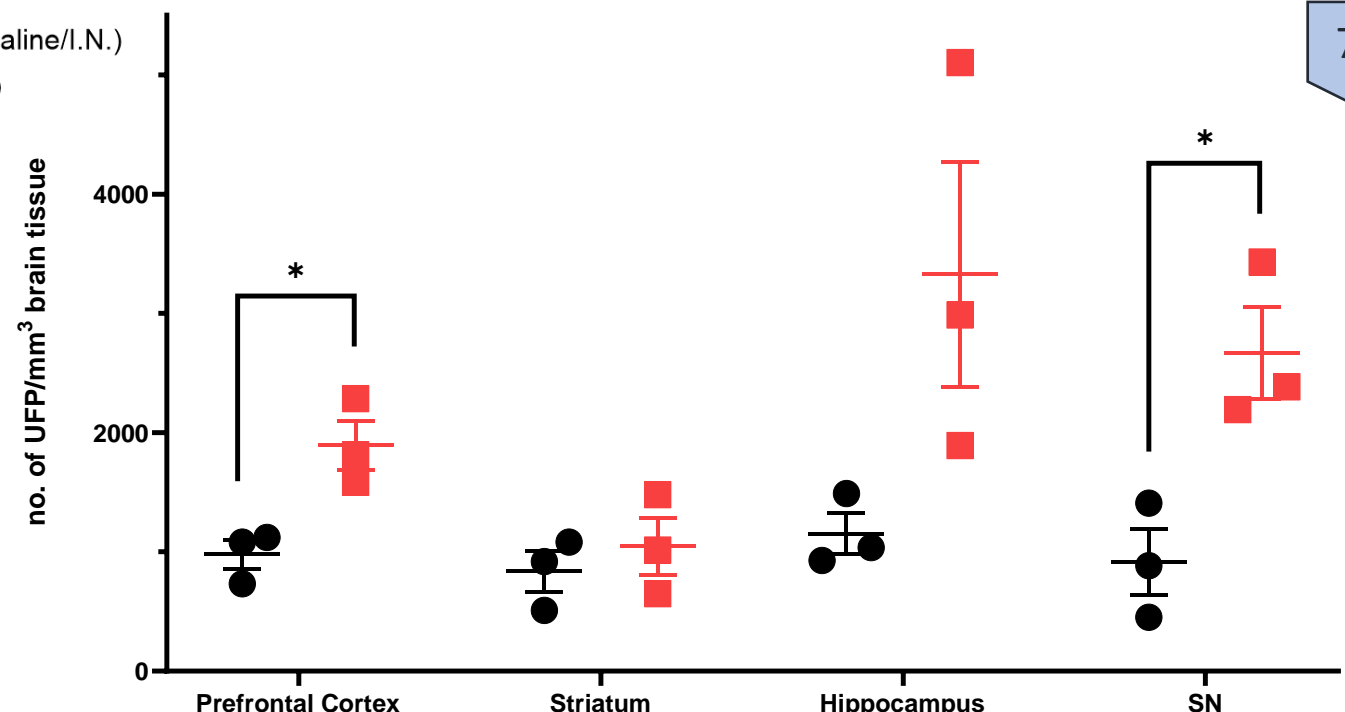
coronal sections
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3 months of
UFP exposure



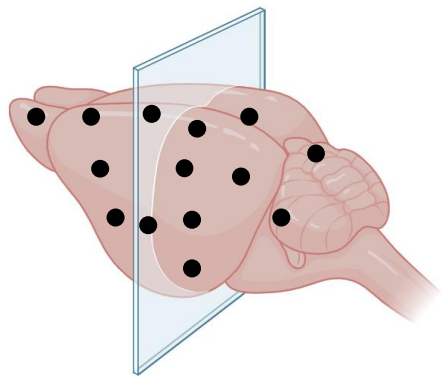
Femtosecond pulsed laser illumination
White light emission

3 months of
UFP exposure
+
3 months of
recovery



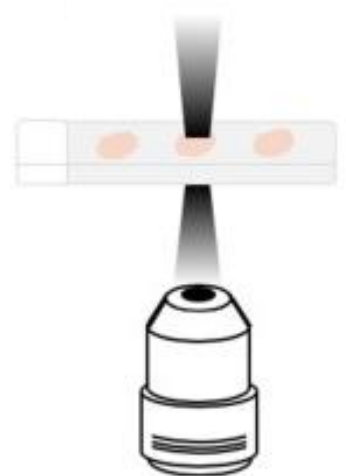
Direct UFP brain measurement

● Control (sterile saline/I.N.)
■ UFP (30 µg/I.N.)



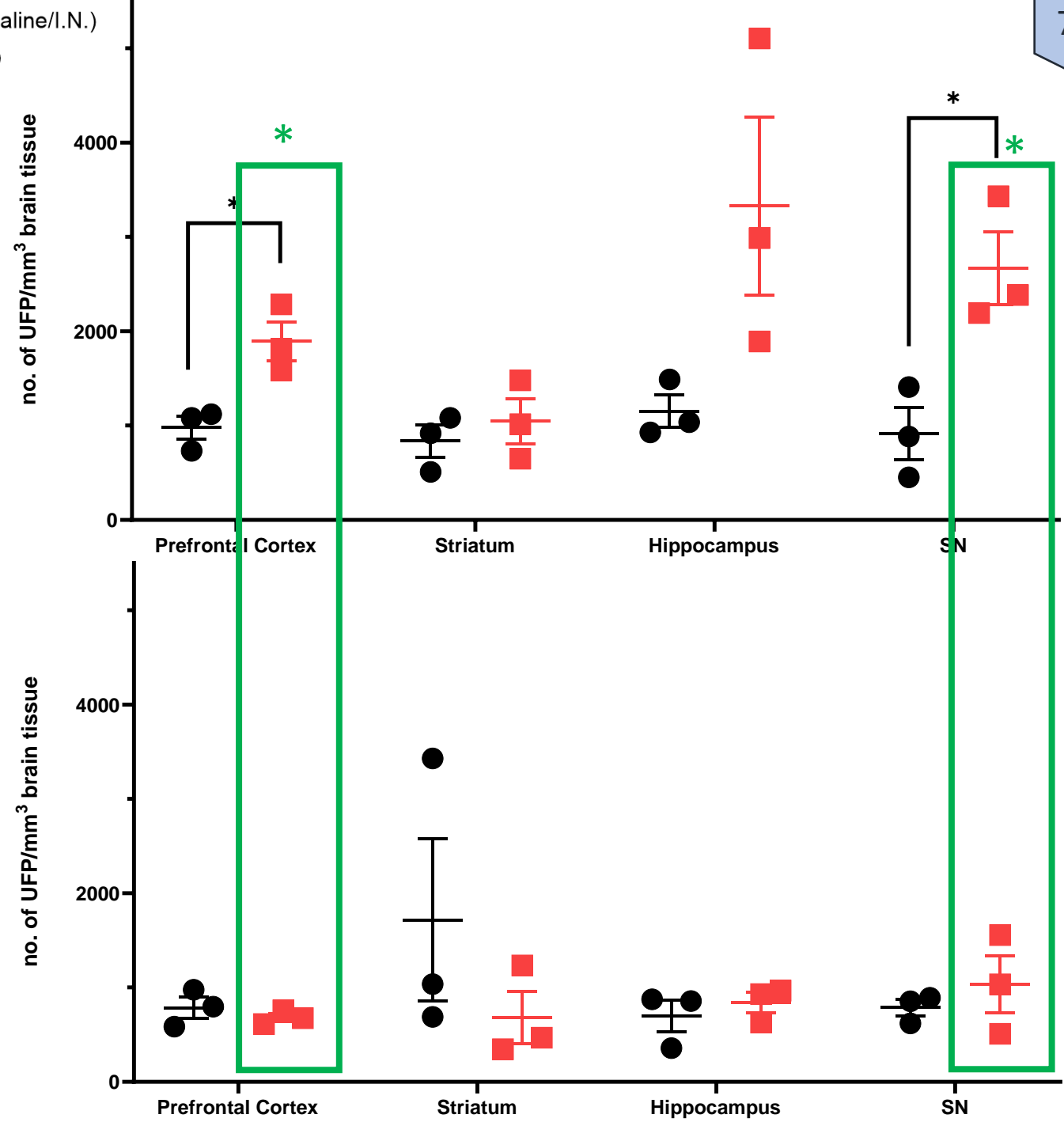
coronal sections
4 brain regions

3 months of
UFP exposure



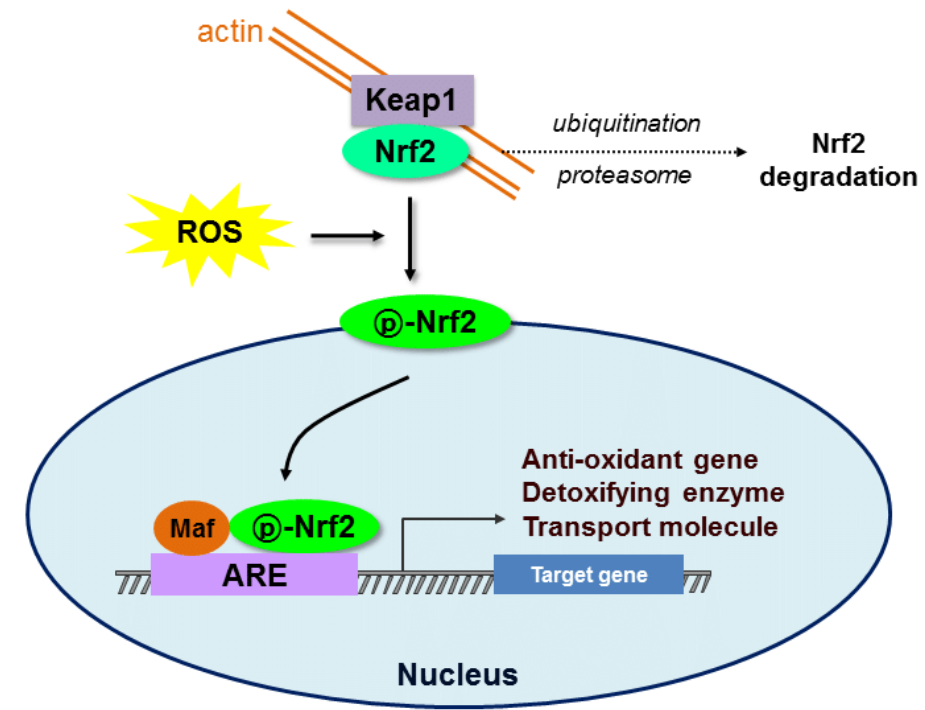
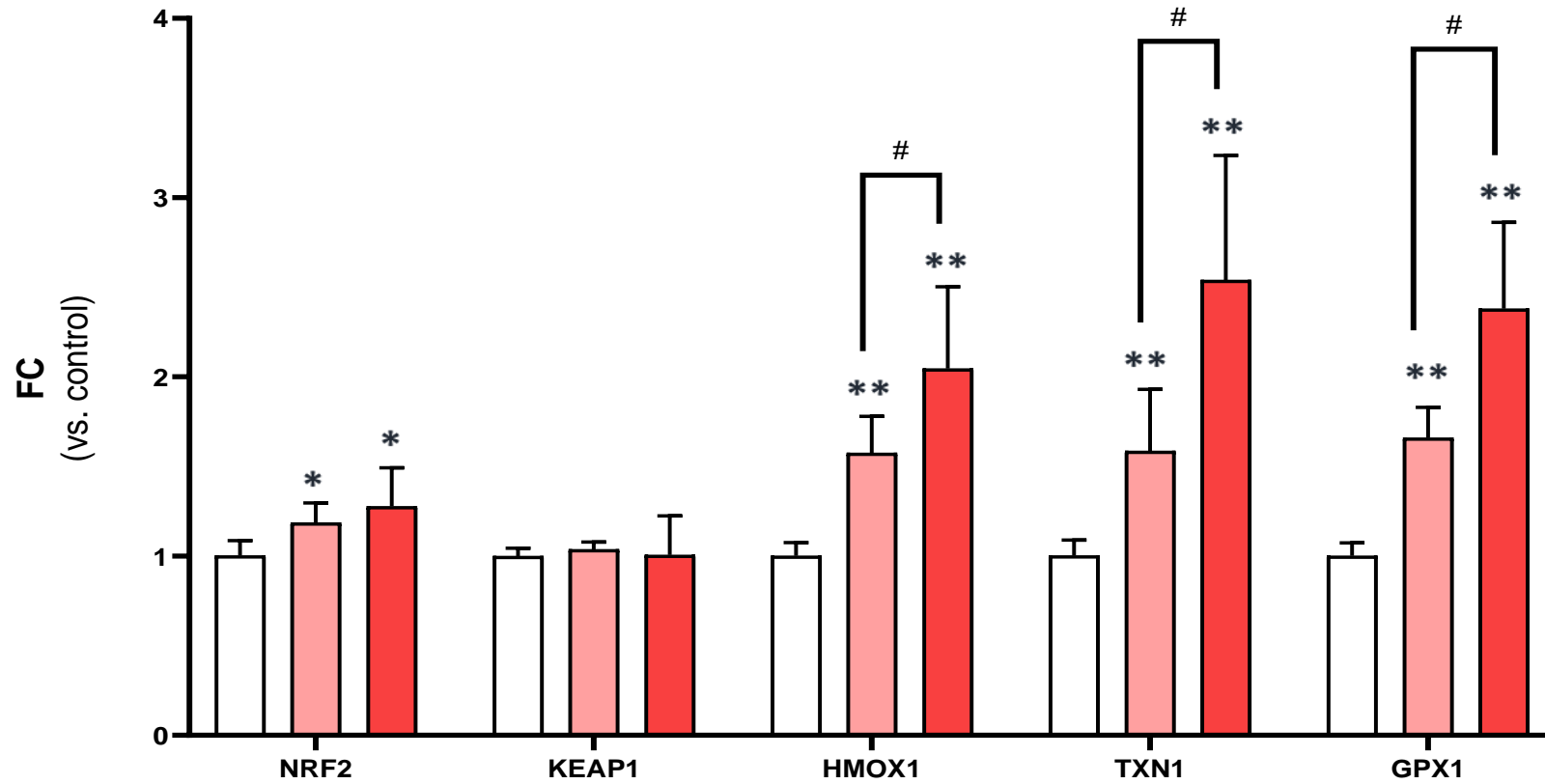
Femtosecond pulsed laser illumination
White light emission

3 months of
UFP exposure
+
3 months of
recovery





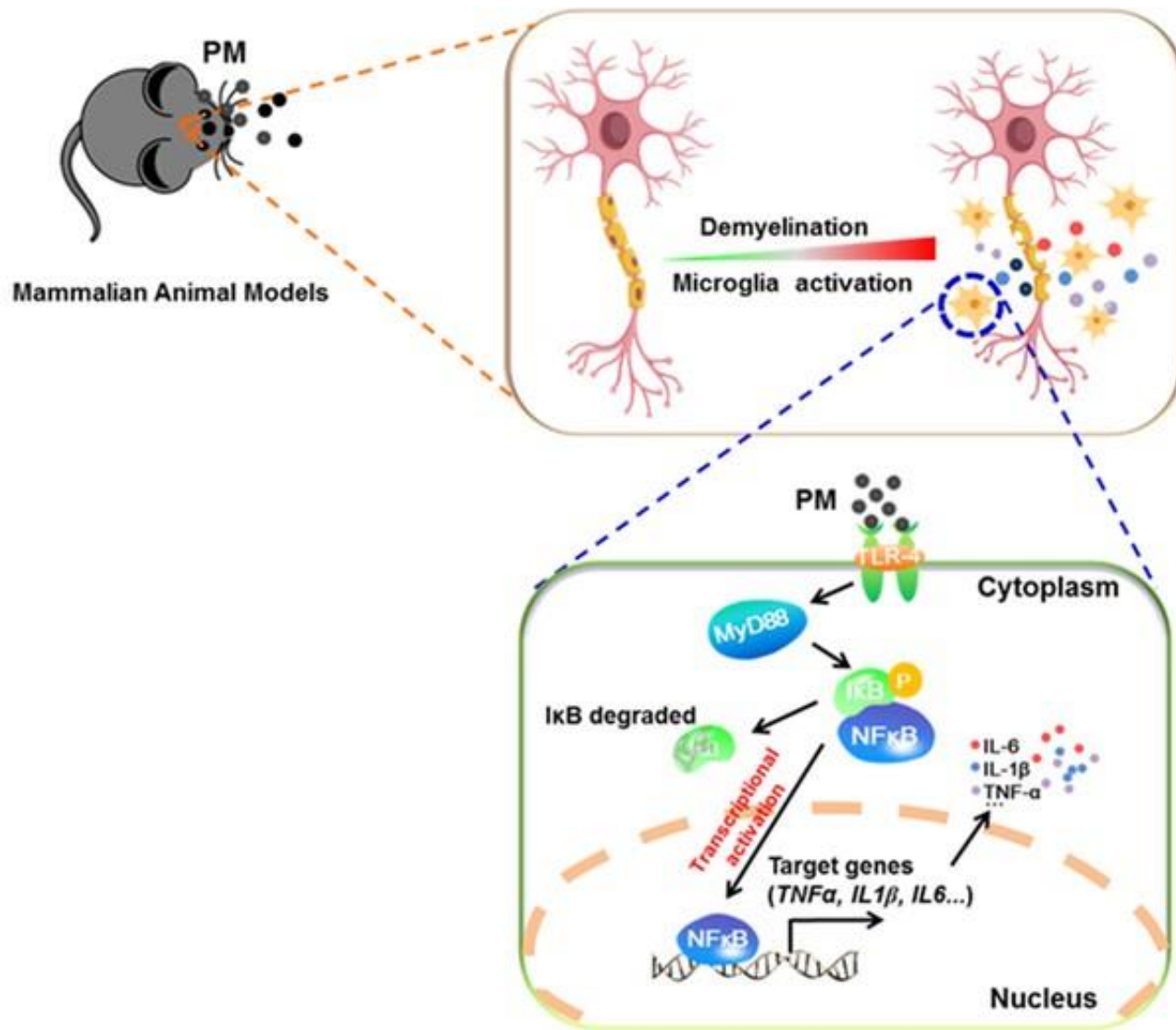
RT-qPCR NRF2 and downstream regulated genes



(Figure of Sin Oh et al., 2017)

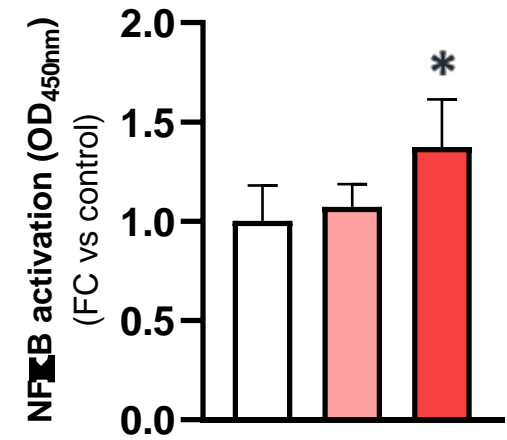
n = 6
 Non-parametric Mann-Whitney U test
 (GraphPad Prism8)
 Statistical analysis: * p<0.05; ** p<0.01.

Nrf2 antioxidant defense pathway and neuroinflammation

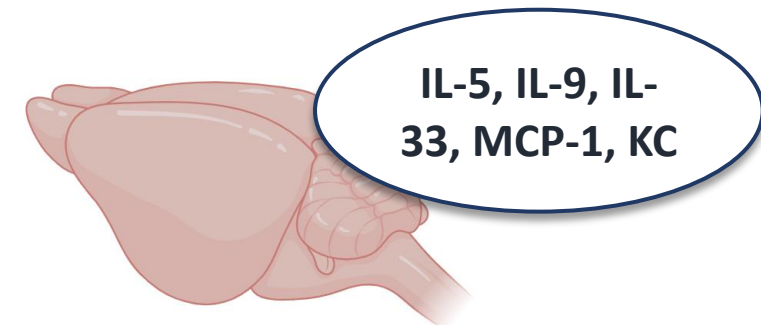


(Figure of Han et al., 2022)

Spectrophotometric optical density DNA affinity binding assay (DAPA) of transcription factor NF κ B



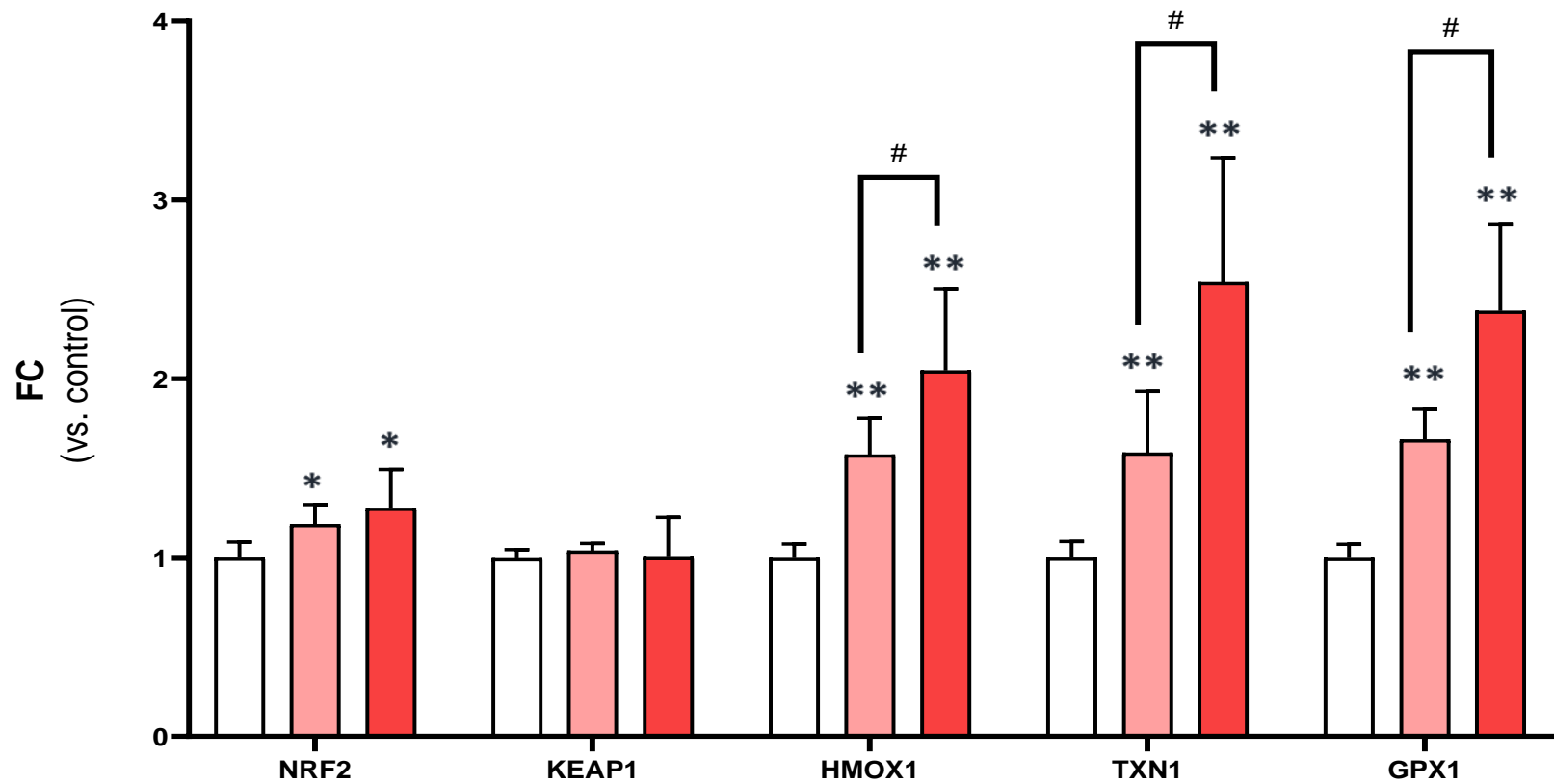
MSD: Proinflammatory and Cytokine Panel (mouse) kits



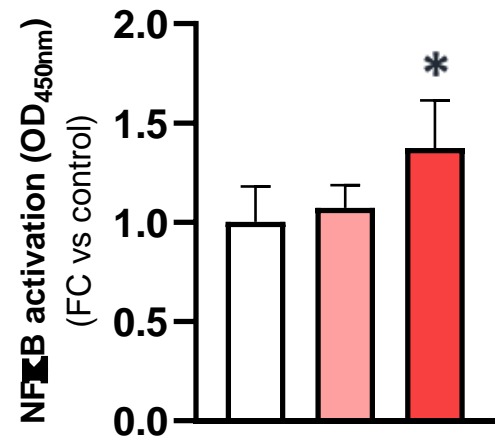
n = 6
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Nrf2 antioxidant defense pathway and neuroinflammation

RT-qPCR NRF2 and downstream regulated genes

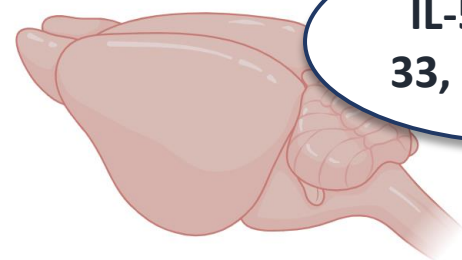


Spectrophotometric optical density DNA affinity binding assay (DAPA) of transcription factor NFκB



MSD: Proinflammatory and Cytokine Panel (mouse) kits

IL-5, IL-9, IL-33, MCP-1, KC



n = 6
Non-parametric Mann-Whitney U test
(GraphPad Prism8)
Statistical analysis: * p<0.05; ** p<0.01.

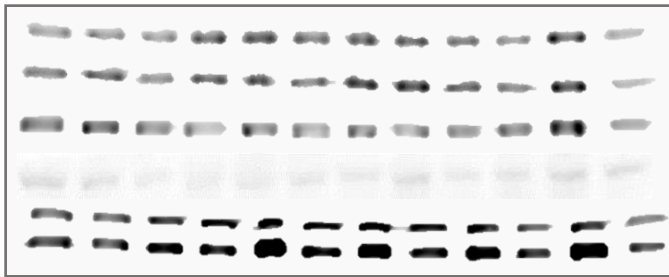
Regulated Cell Death (RCD)

Apoptosis

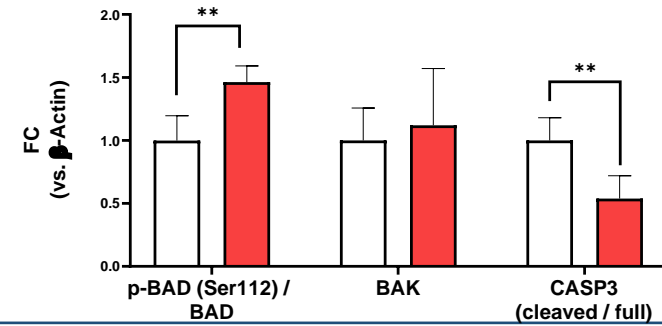
Control (n=6) 30µg UFP (n=6)

Pro-apoptosis

Caspase cascade



BAD
Phospho-BAD (Ser112)
BAK
Full Casp3 (35 kDa)
Cleaved Casp3 (19 kDa)
Cleaved Casp3 (17 kDa)

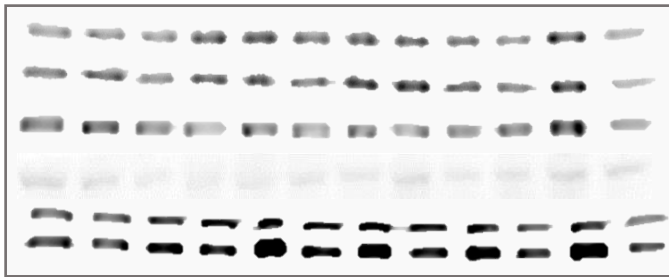


Regulated Cell Death (RCD)

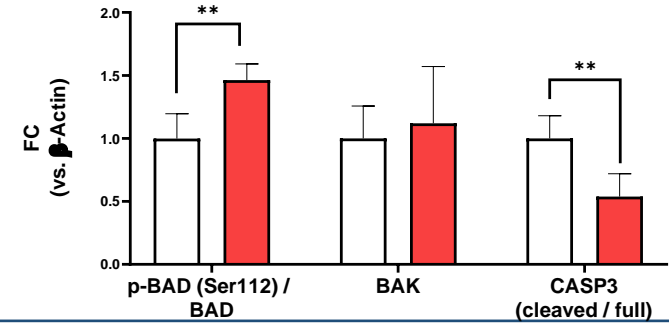
Apoptosis

Control (n=6) 30µg UFP (n=6)

Pro-apoptosis
Caspase cascade

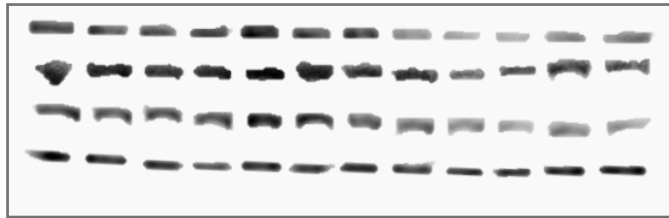


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Phospho-BAD (Ser112)
BAK
Full Casp3 (35 kDa)
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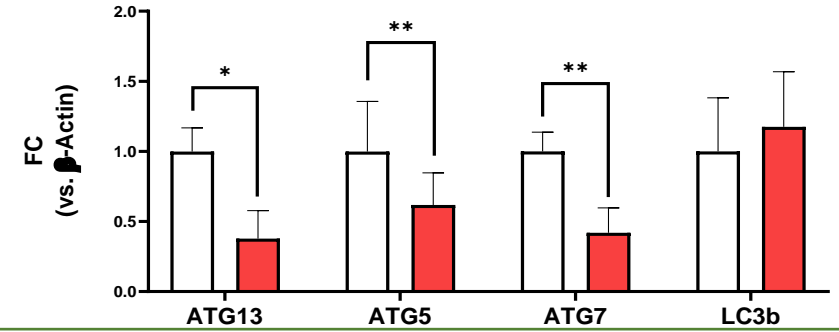


Autophagy

Autophagosome formation



ATG13
ATG5
ATG7
LC3b



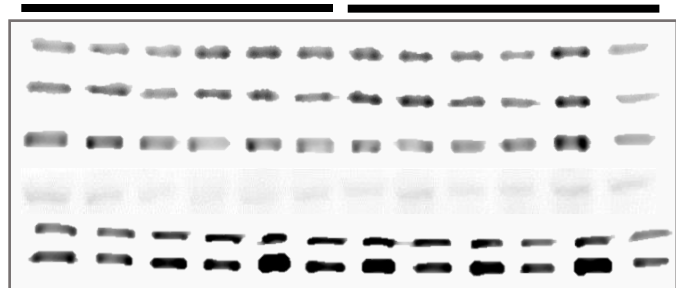
Regulated Cell Death (RCD)

Apoptosis

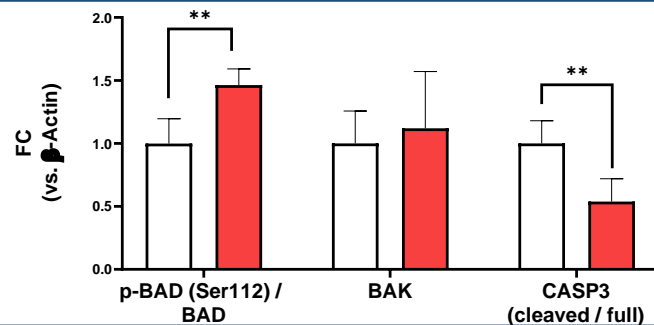
Control (n=6) 30µg UFP (n=6)

Pro-apoptosis

Caspase cascade

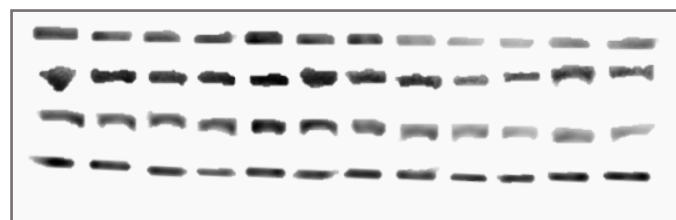


BAD
Phospho-BAD (Ser112)
BAK
Full Casp3 (35 kDa)
Cleaved Casp3 (19 kDa)
Cleaved Casp3 (17 kDa)

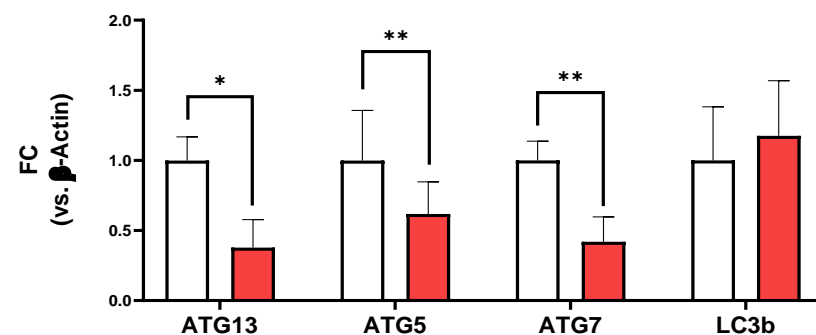


Autophagy

Autophagosome formation



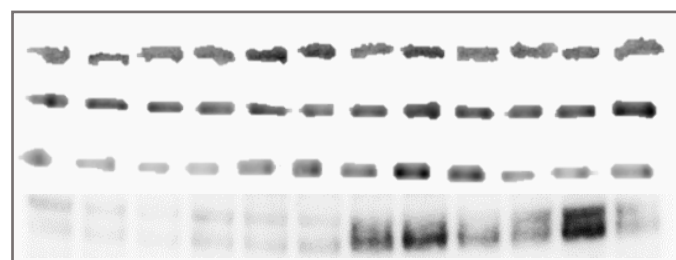
ATG13
ATG5
ATG7
LC3b



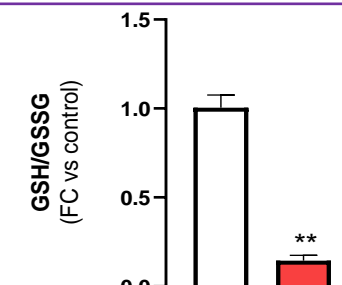
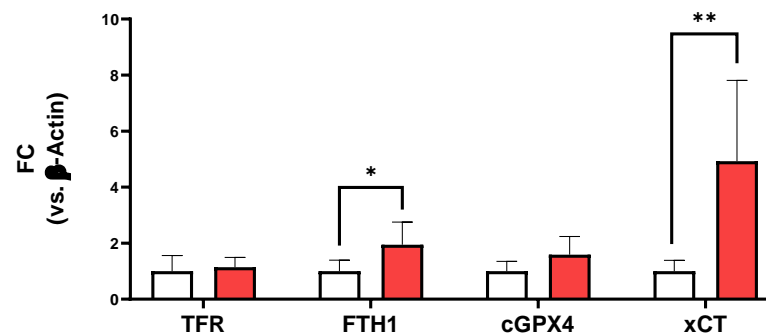
Ferroptosis

Iron imbalance

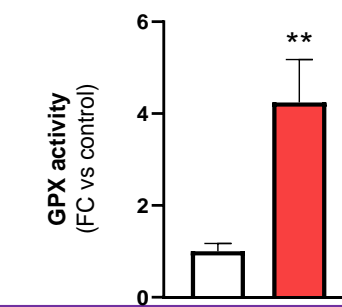
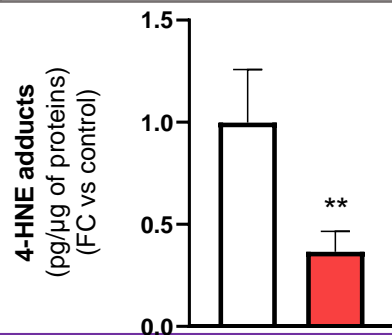
GSH-antioxidant defense



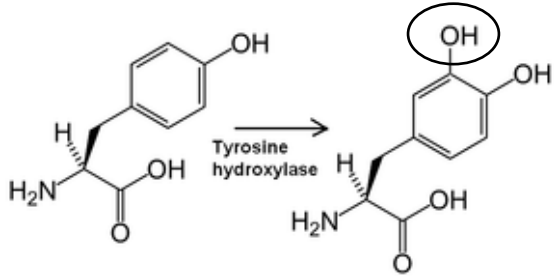
TFR
FTH1
cGPX4
xCT



Lipid peroxidation



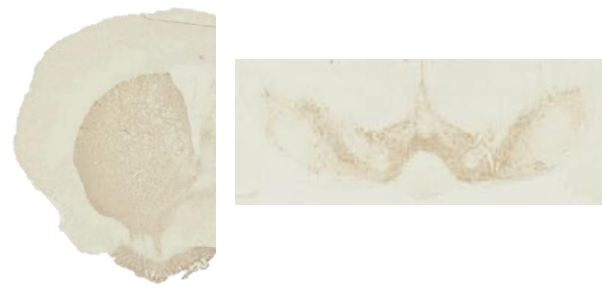
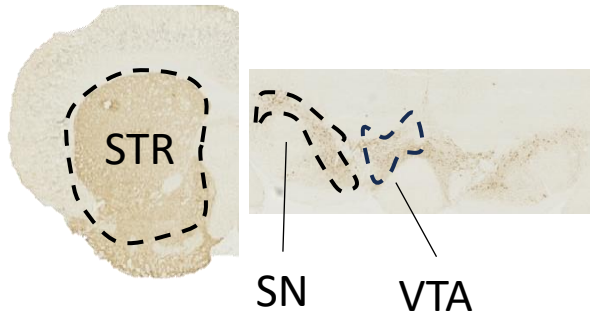
Specific neurodegeneration in brain sections



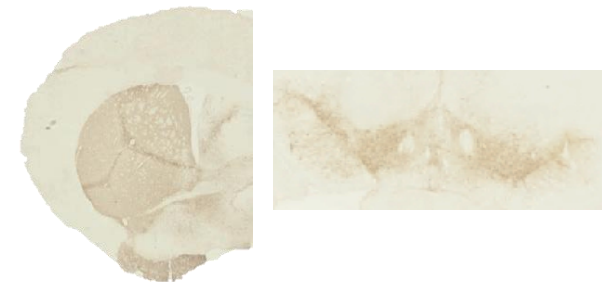
3 months of UFP exposure

3 months of UFP exposure + 3 months of recovery

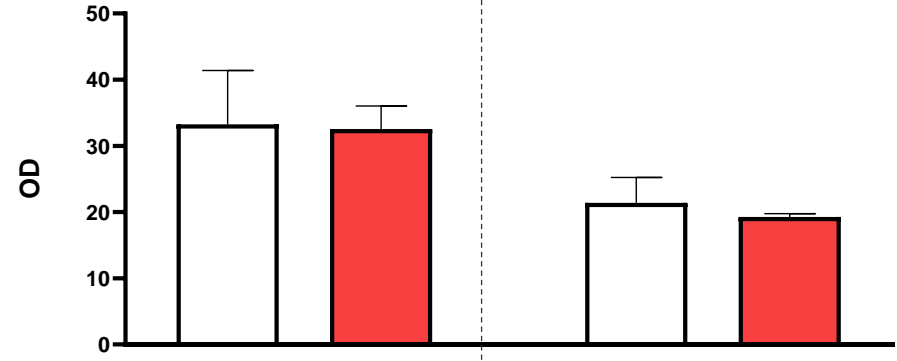
Control



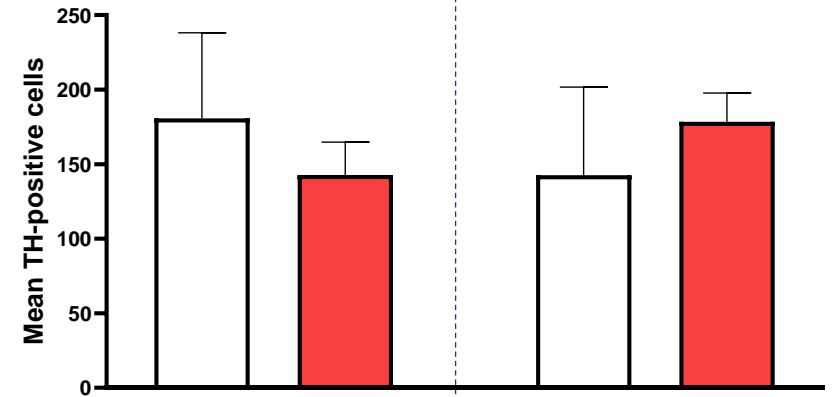
30 µg/adm UFP



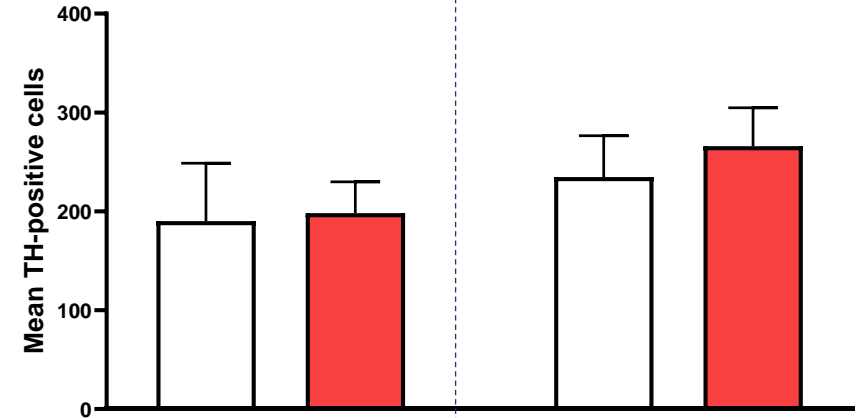
Striatum



SN



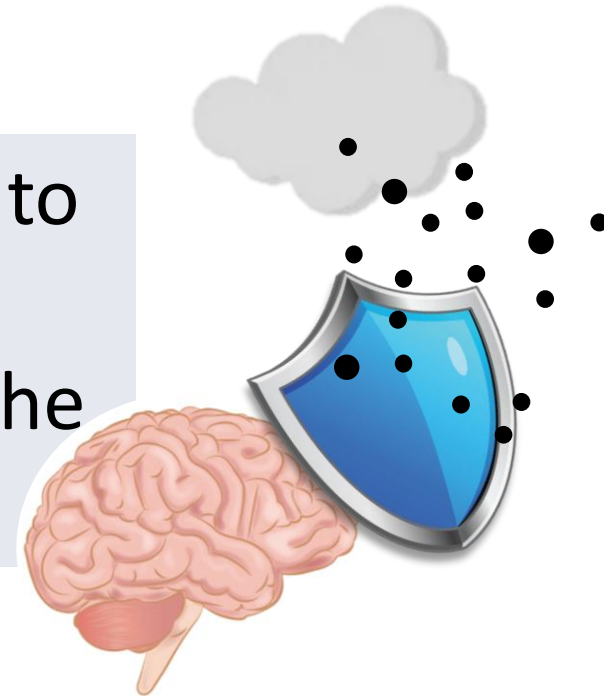
VTA





- **No behavioral changes in exposed-mice**
- **UFP reached the brain**
- **Induction NRF2 pathway and NFκB**
- **RCD repression in (whole) brain tissue**

Male BALB/c mice **postnatal subchronically exposed** to UFP led to adaptative protection. Results show the combativity to environmental air-pollution toxins of the brain.



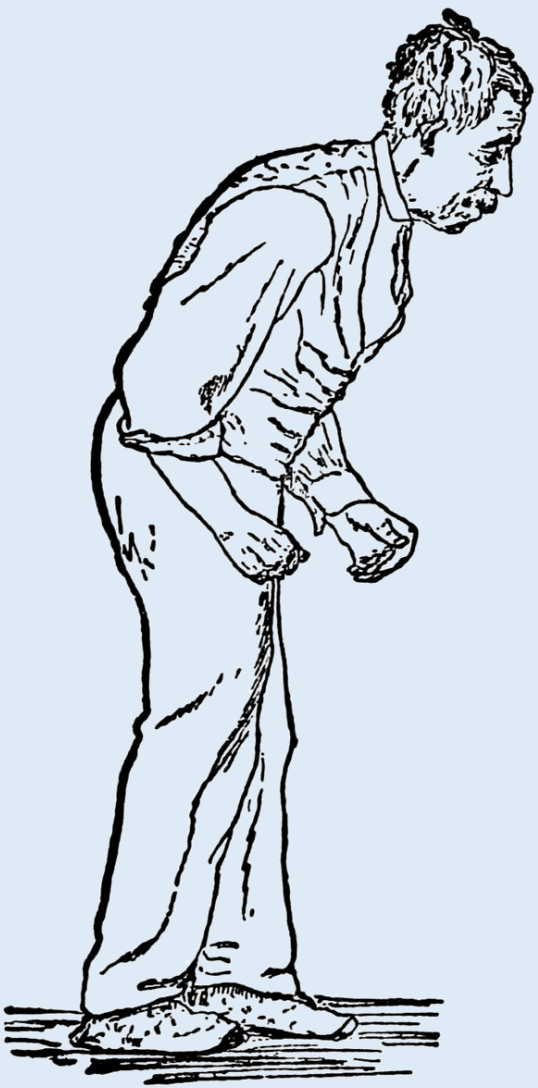


- **No behavioral changes in exposed-mice**
- **UFP reached the brain**
- **Induction NRF2 pathway and NFκB**
- **RCD repression in (whole) brain tissue**

Specific zones (prefrontal cortex, SN)
+
Cellular defense & neuroinflammation
+
Decreased autophagy → α-synuclein aggregation ?
+
Iron accumulation (FTH1 ↗)

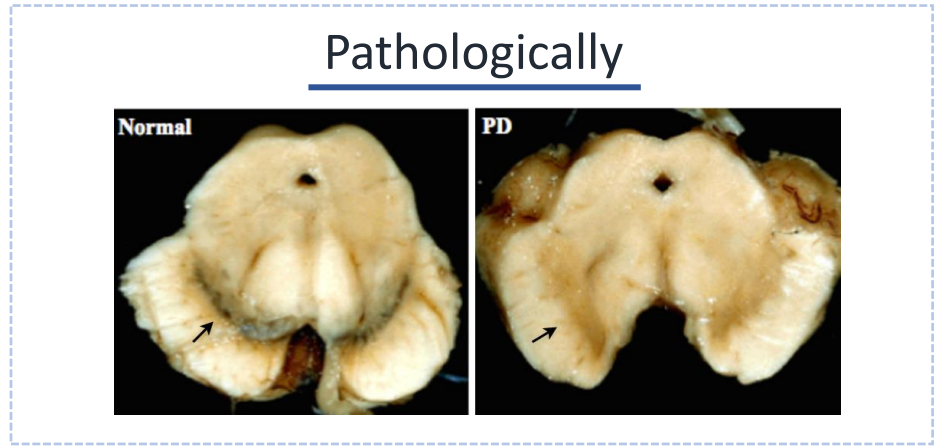
Parkinson's disease

- Complex neuropsychiatric
- Second most common neurodegenerative disease
- > 10 million people worldwide



<u>Clinically</u>	
Motor symptoms	Non-motor symptoms
Bradykinesia	Multiple variable
Resting tremor	cognitive symptoms
Rigidity	depression/anxiety
Postural instability	dysautonomia

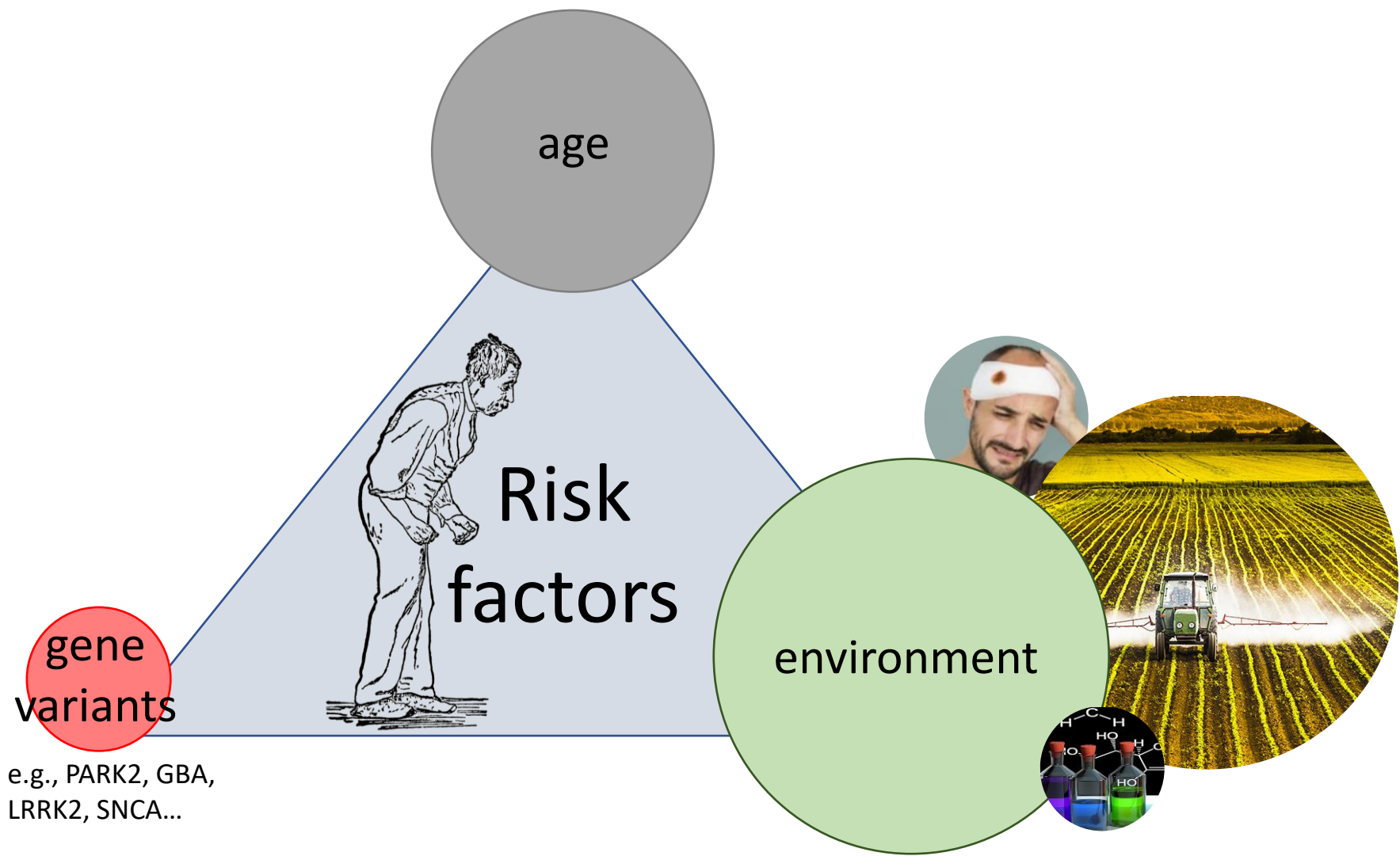
- Cellular and molecular mechanisms
- Aggregation α -syn
 - Oxidative stress
 - Lipid peroxidation
 - Neuroinflammation
 - Iron accumulation



- Cell death types
- Apoptosis
 - Autophagy
 - **Ferroptosis**

(Guiney *et al.*, 2017)

Interplay of aging, genes and environment



UFP



High Volume Impactor Sampler

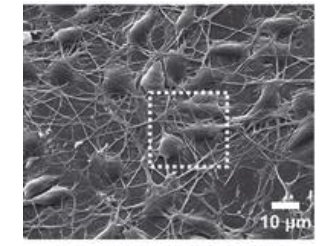
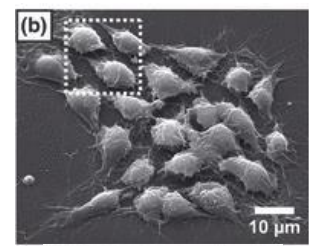
Autumn/Winter 2014

chemical composition:
e.g., Fe: 10.267 mg/g

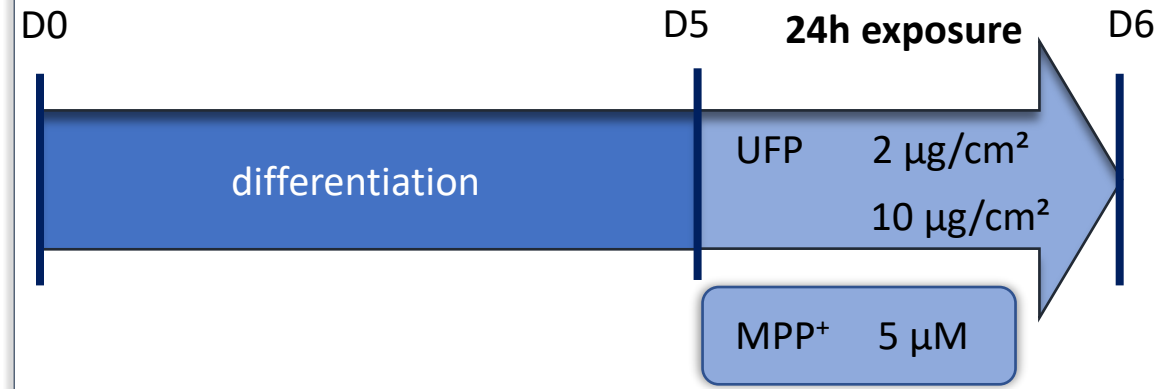
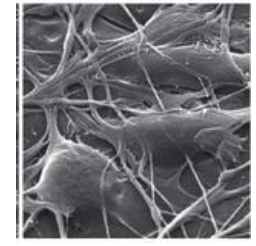
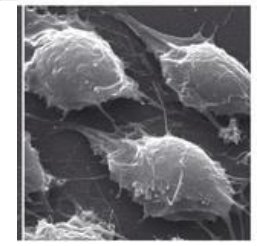
- Industry and port
- ± 44 km²
- 86,788 inhabitants

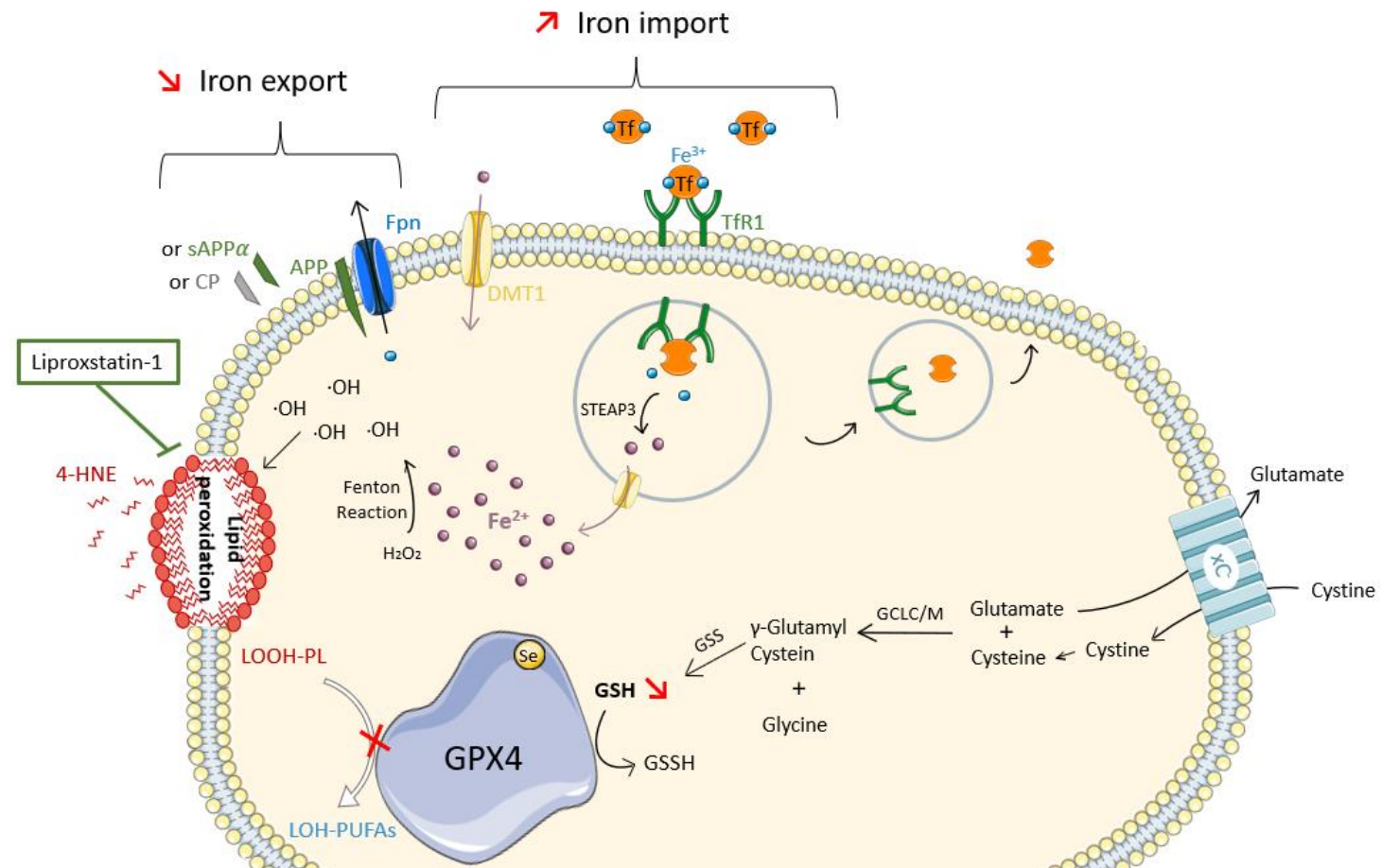


EXPOSURE

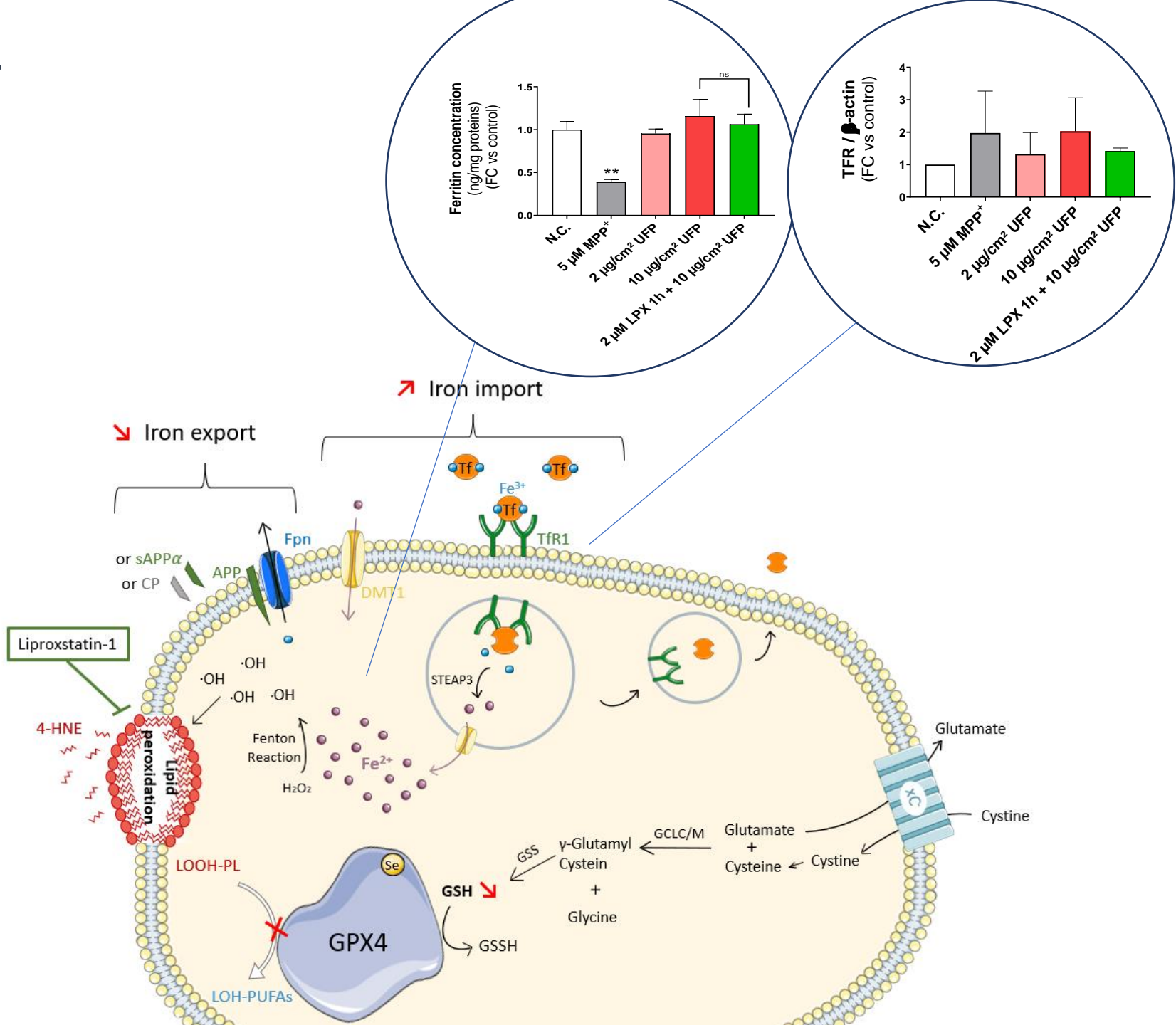


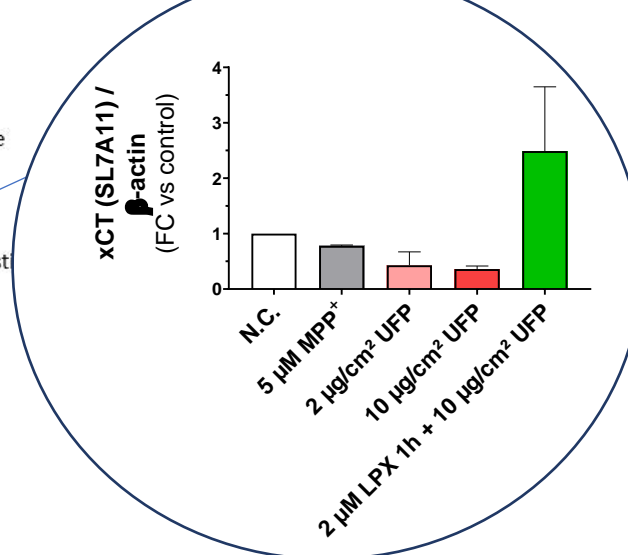
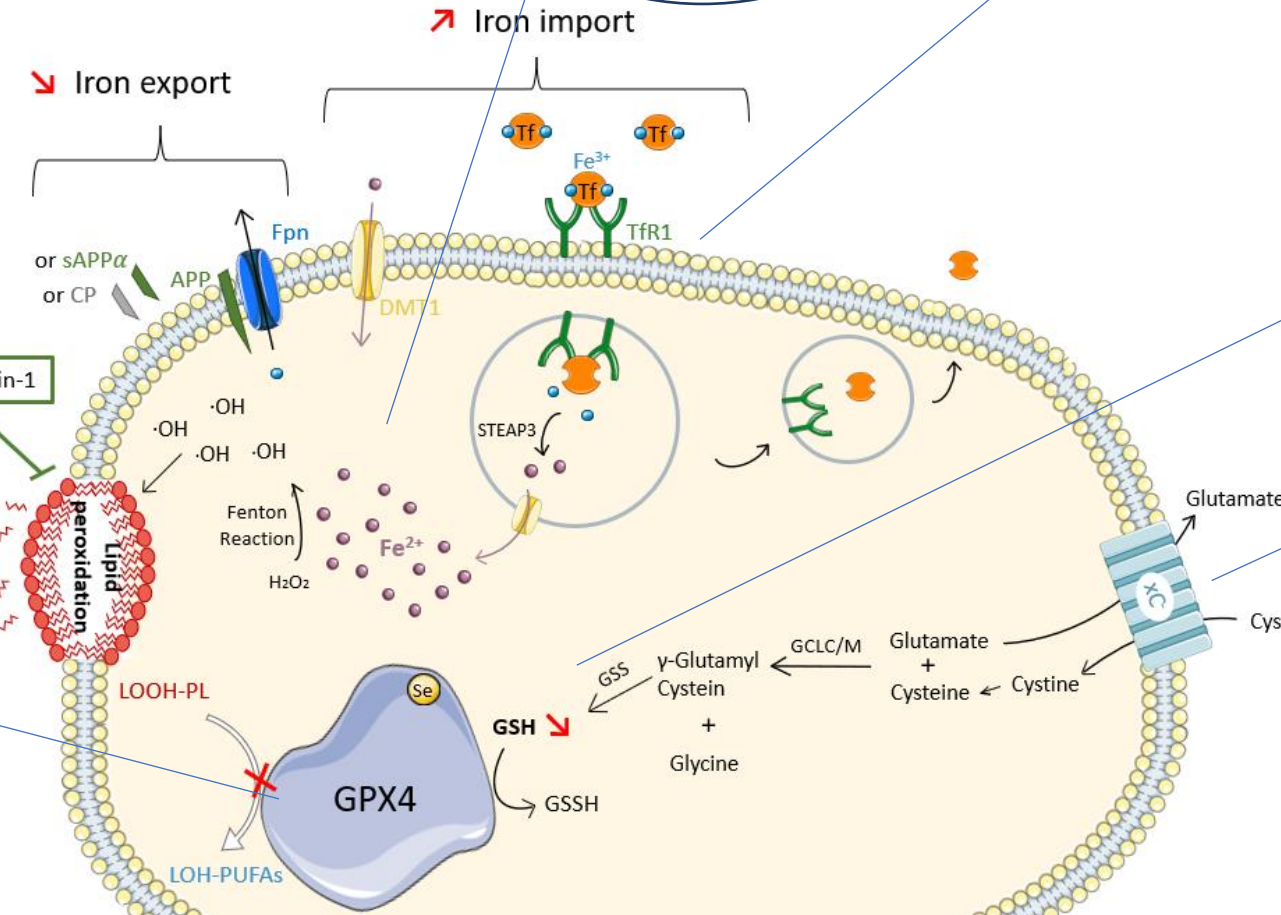
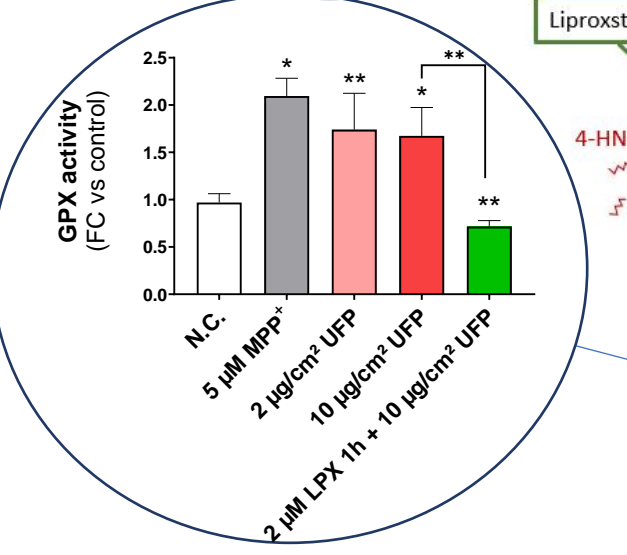
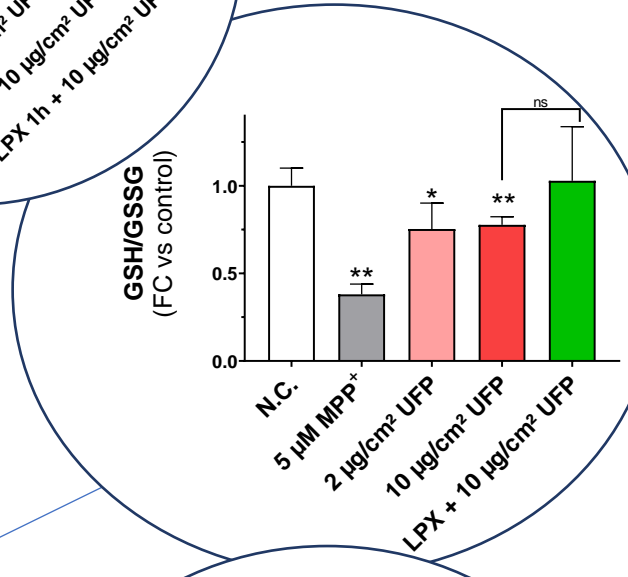
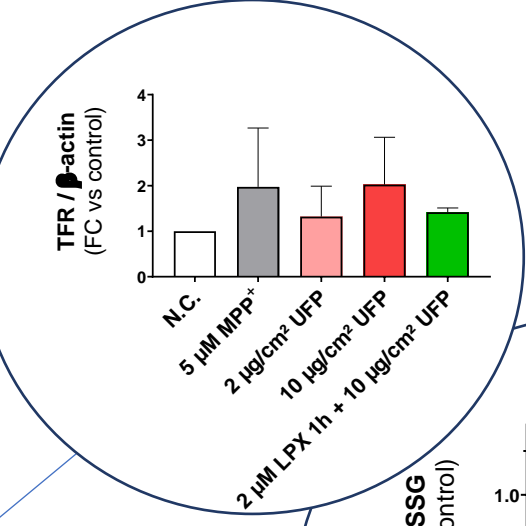
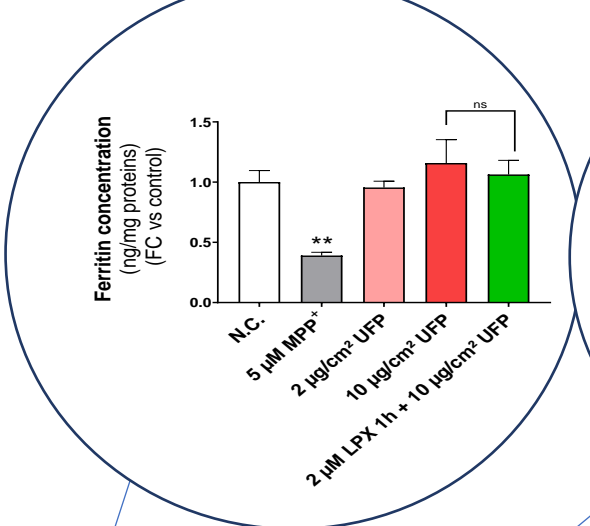
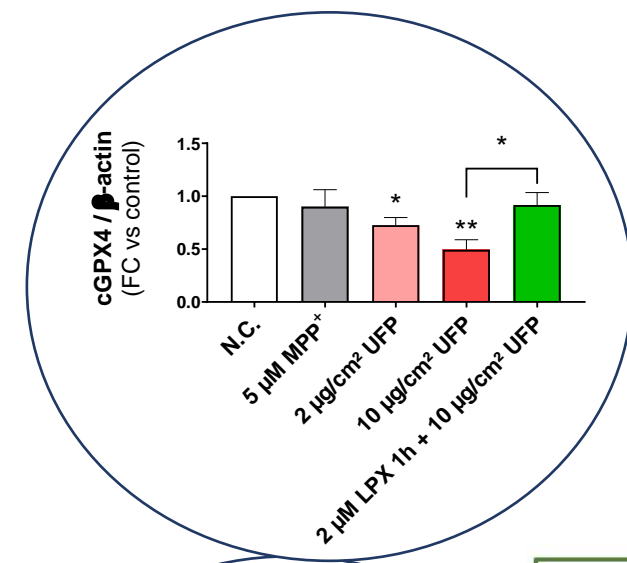
mature dopaminergic neurons

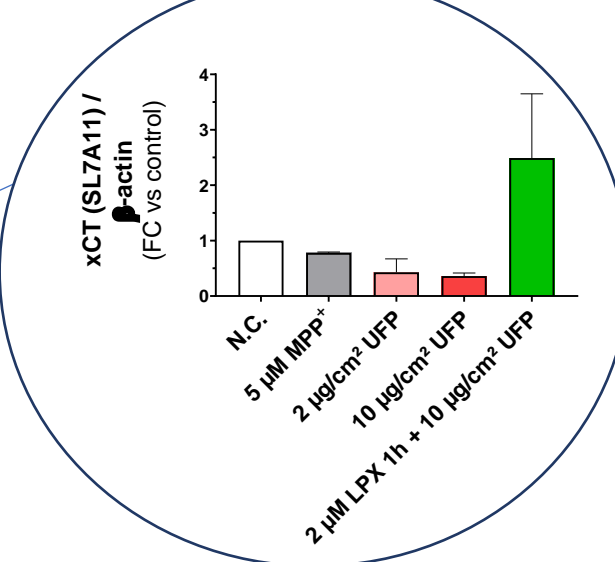
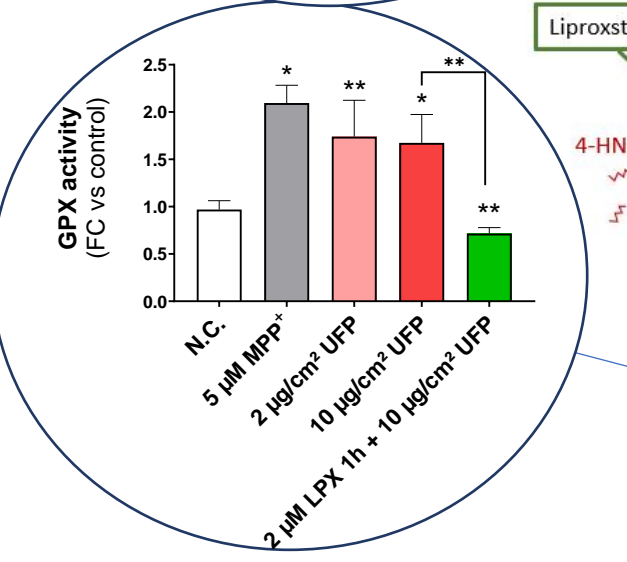
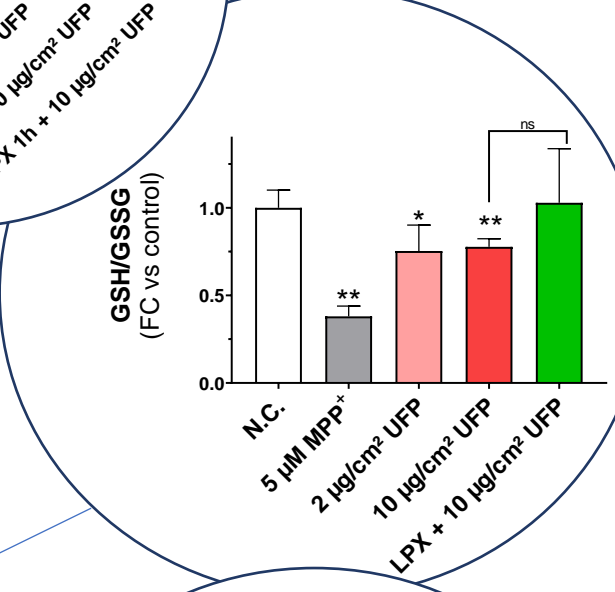
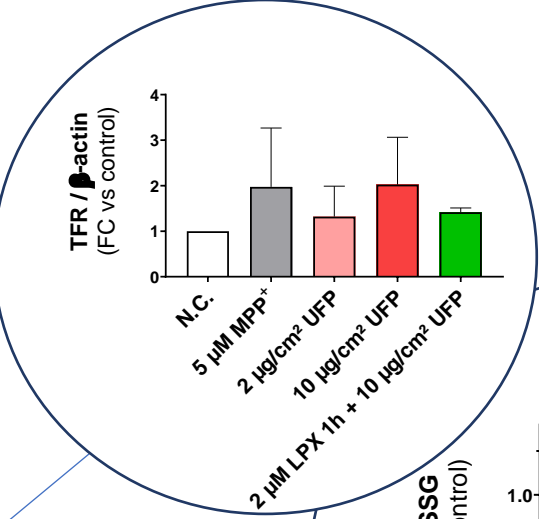
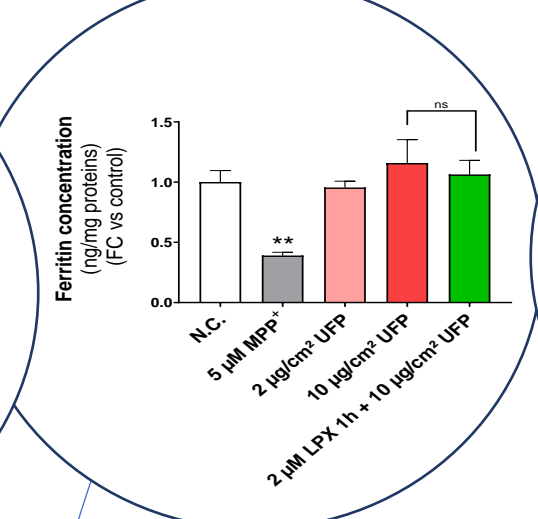
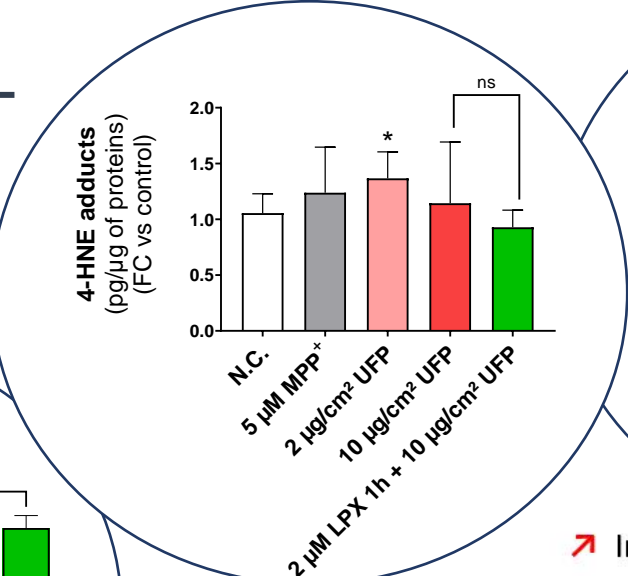
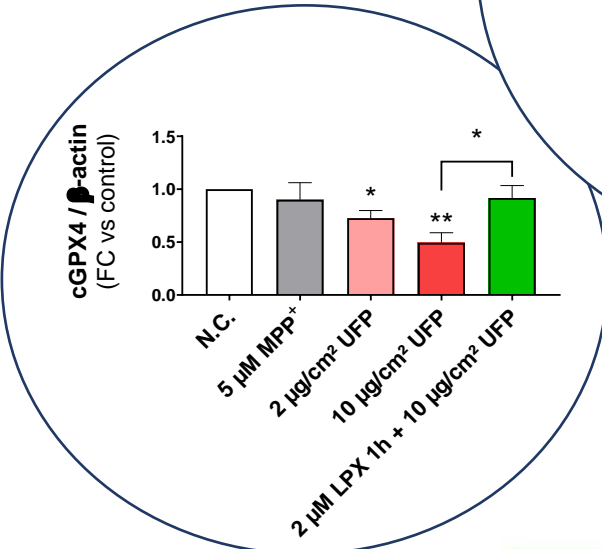
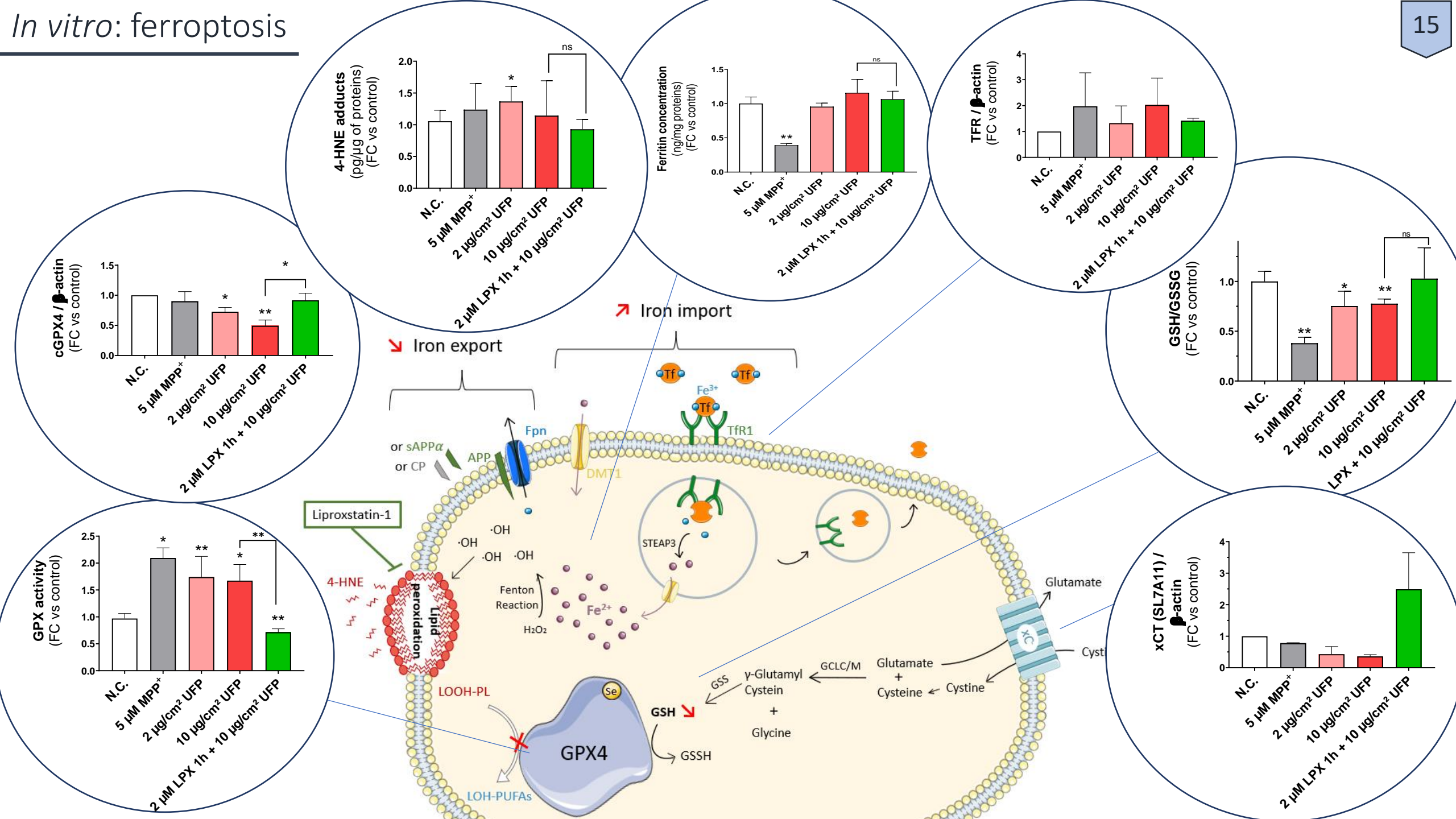


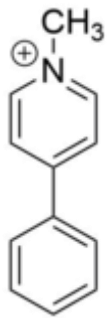


In vitro: ferroptosis

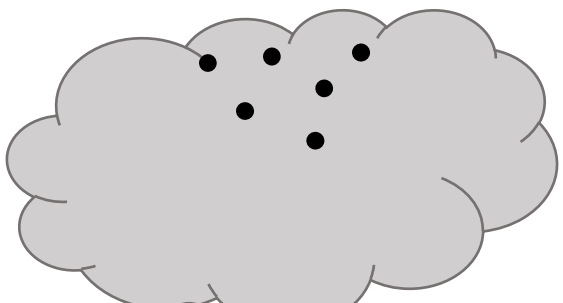
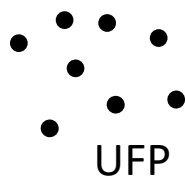




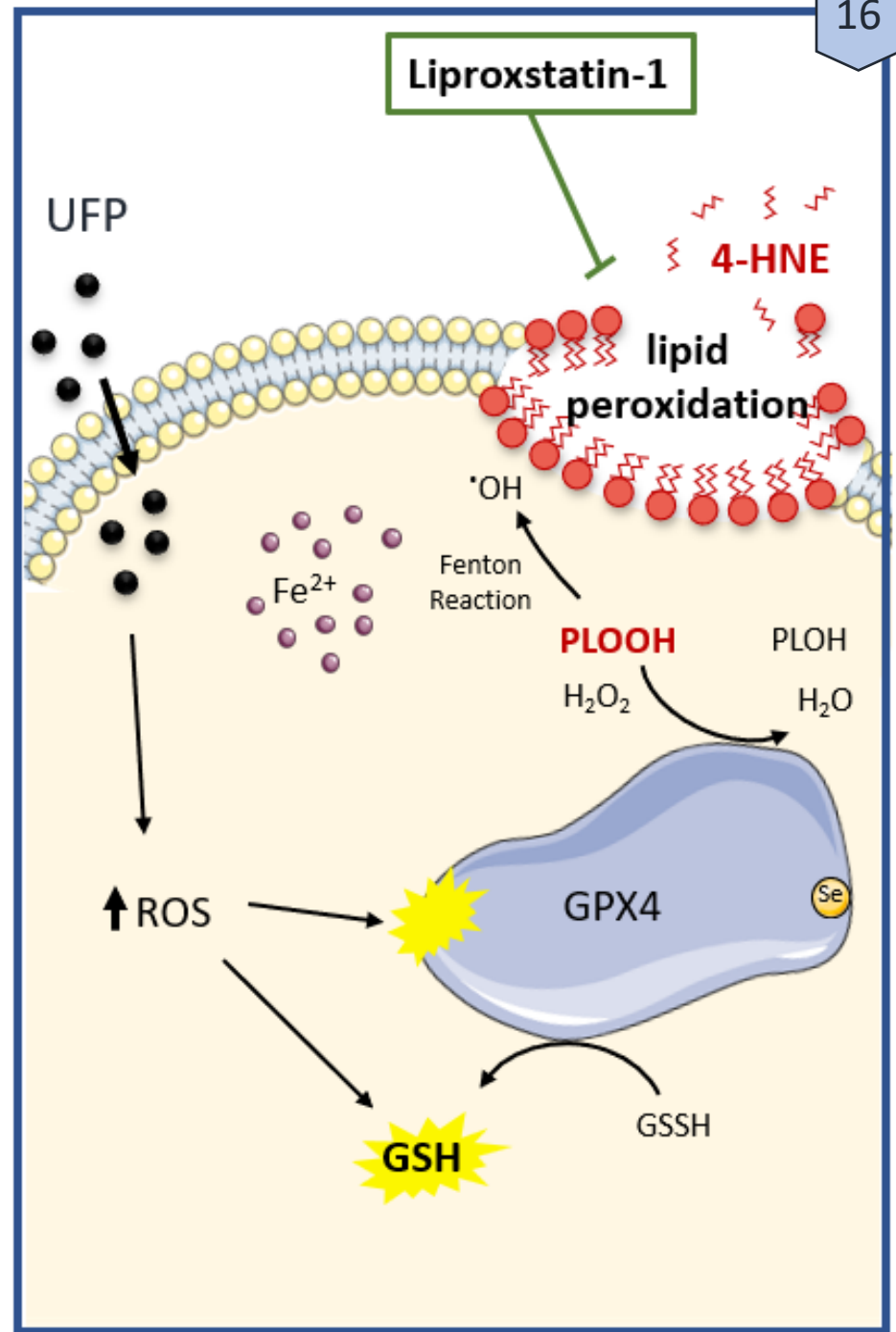




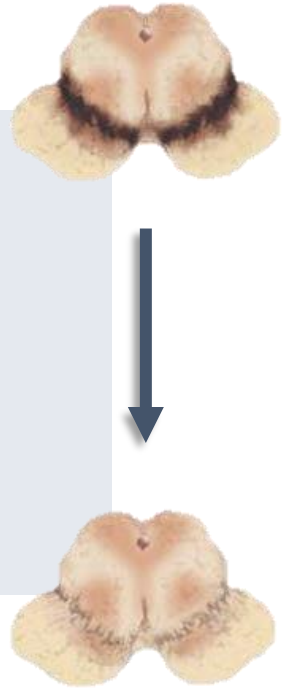
MPP⁺



UFP could contribute to the development of a PD-phenotype in LUHMES cells by inducing ferroptosis



In vivo and *in vitro* results suggests that **long-term exposure** to UFP could represent an additional environmental cause of Parkinson's disease through induction of ferroptotic cell death



Thank you !

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Ultrafine Particles – Air Quality and Climate

