Enduring neurotoxic effects of perinatal exposure to pesticides Paraquat/Maneb in mice

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LABORATORY OF EXPERIMENTAL NEUROPATHOLOGY

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1817
(200 years ago)

Parkinson's Disease (PD)

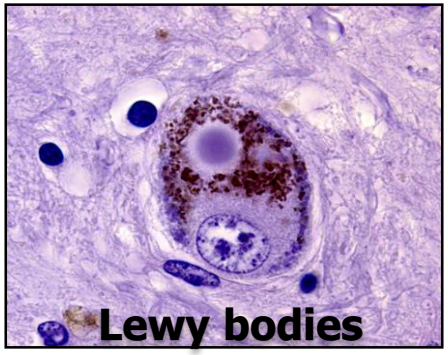
- Second most common neurodegenerative disorder;
- Symptomatology: motor and non-motor symptoms

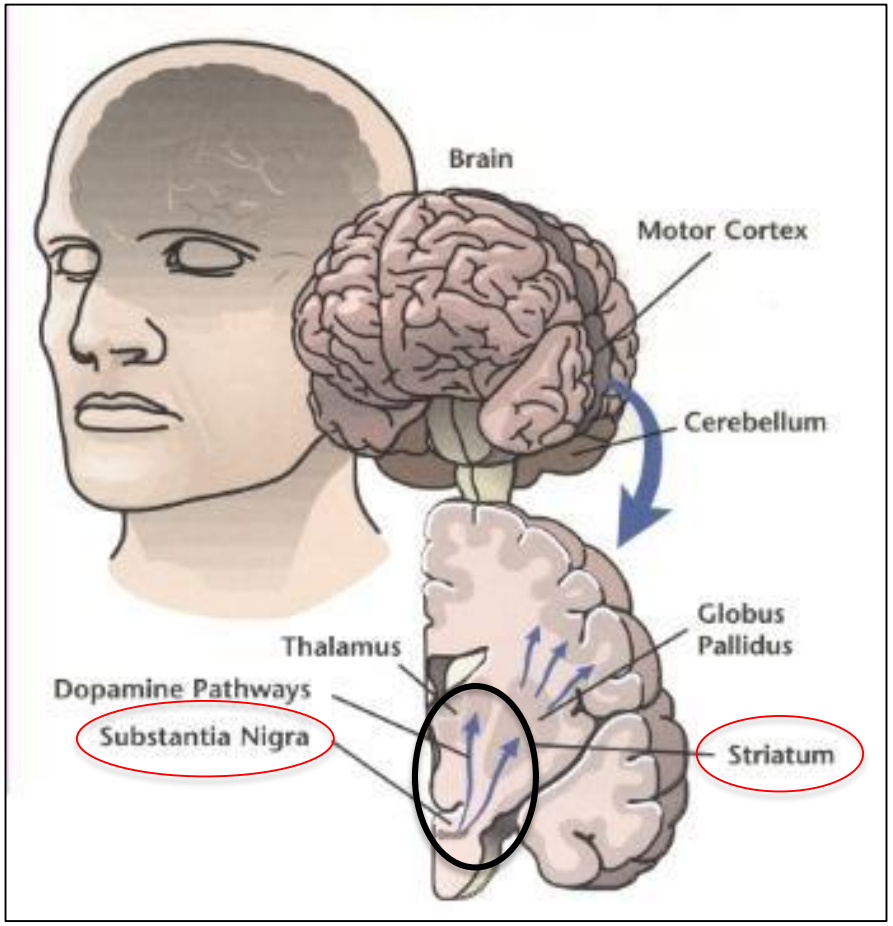
HIRSCH et al., 2013, MOV DISORD

Major neuropathological aspects

- Loss of dopaminergic neurons particularly in the substantia nigra pars compacta, which leads to loss of dopaminergic terminals in the striatum;

- Presence of Lewy bodies (abnormal aggregates of proteins)





DAWSON & DAWSON, 2003, SCIENCE

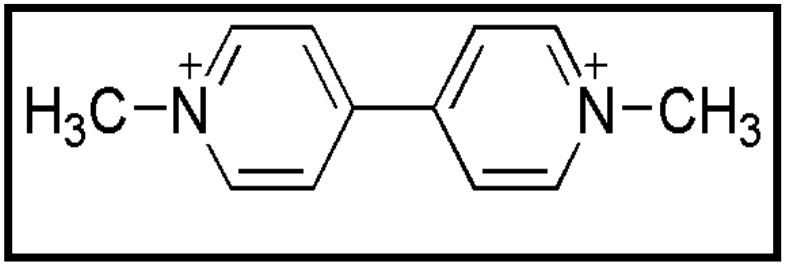
Parkinson's Disease vs. Pesticide Exposure

- Experimental data shows that specific pesticides causes PD-related symptoms and neurochemical changes in animals;
- Epidemiological data with **humans** show that PD is positively associated with pesticide exposure.

CORY-SLECHTA et al., 2005, EHP; TANNER et al., 2011, EHP

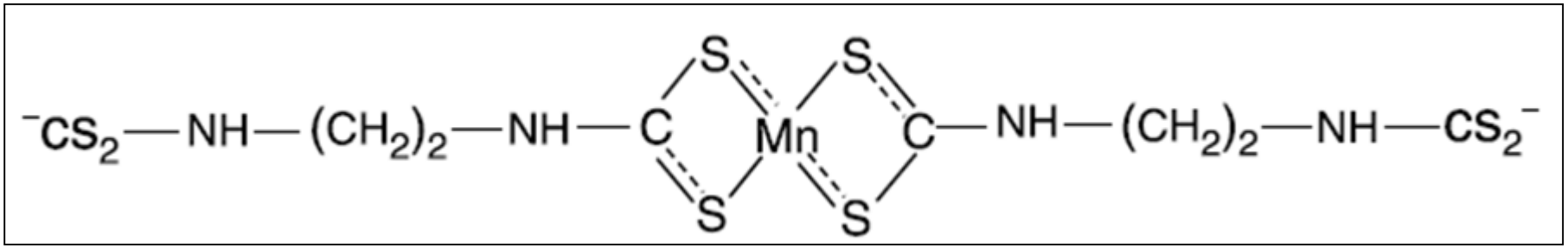
Paraquat (PQ)

- Herbicide highly used in Brazil;
- Class 2, moderately toxic;
- Causes nigrostriatal neurodegeneration in experimental models;
- Exposure to PQ (**in humans**) was associated with increase in risk of PD.



PEZZOLI & CEREDA., 2013, NEUROLOGY

Maneb (MB)



- A widely used fungicide
- Causes motor symptoms of PD in experimental animals
- Chronic exposure of **humans** to MB has been linked to the development of Parkinsonism

MORATO et alL., 1989, NEUROTOXICOL TERATOL; FERRAZ et al., 1988, NEUROLOGY

PQ/MB – GEOGRAFICAL OVERLAP

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Original Contribution

Parkinson's Disease and Residential Exposure to Maneb and Paraquat From Agricultural Applications in the Central Valley of California

Sadie Costello, Myles Cockburn, Jeff Bronstein, Xinbo Zhang, and Beate Ritz

"… exposure to a combination of MB and PQ increases PD risk, particularly in younger subjects and/or **when exposure occurs at younger ages**".

OBJECTIVES

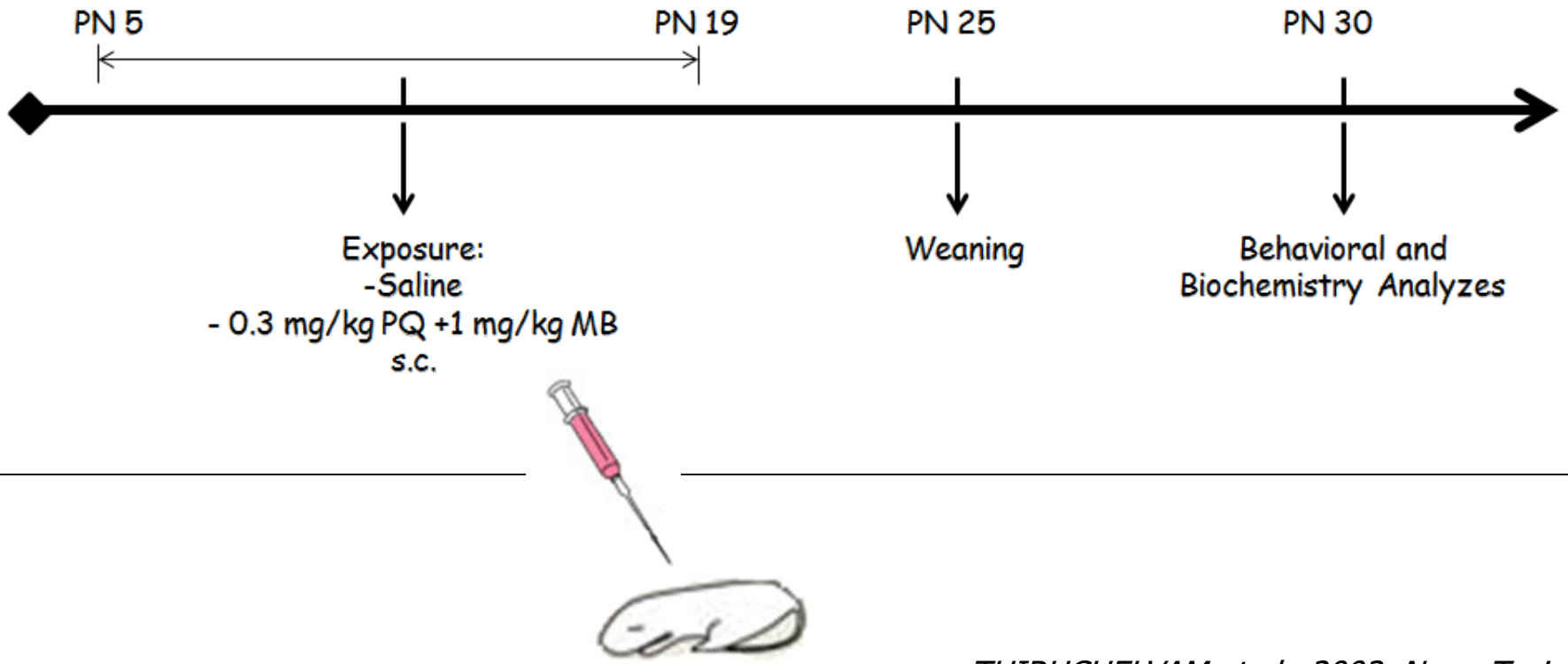
- Investigate whether **early postnatal** PQ/MB exposure would produce persistent neurotoxic effects in mice (focus on nigrostriatal system);
- Investigate whether early postnatal PQ/MB exposure would enhanced adult susceptibility to re-challenge with these same pesticides.

Early postnatal exposure

Early postnatal exposure

- Male Swiss mice: exposed to PQ + MB
- Protocol number: PP00765/CEUA/UFSC

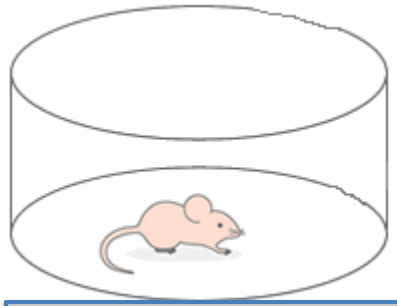
Control **PQ + MB**



THIRUCHELVAM et al., 2002, NeuroToxicol

Early postnatal exposure

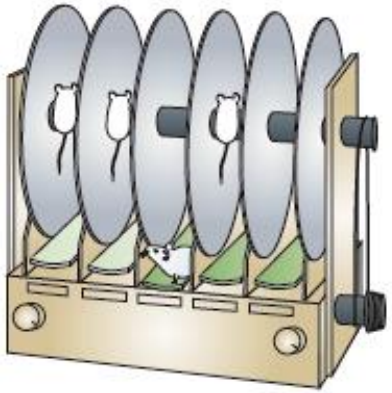
- Behavioral analyses (motor-related parameters)



Open field

(SANTOS et al., 2012)

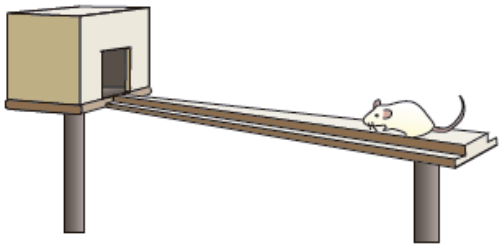
Locomotor and exploratory activities



Rotarod

(KHARIV et al., 2013)

Motor performance



Beam walking

(KHARIV et al., 2013)

Coordination and balance

Pole test

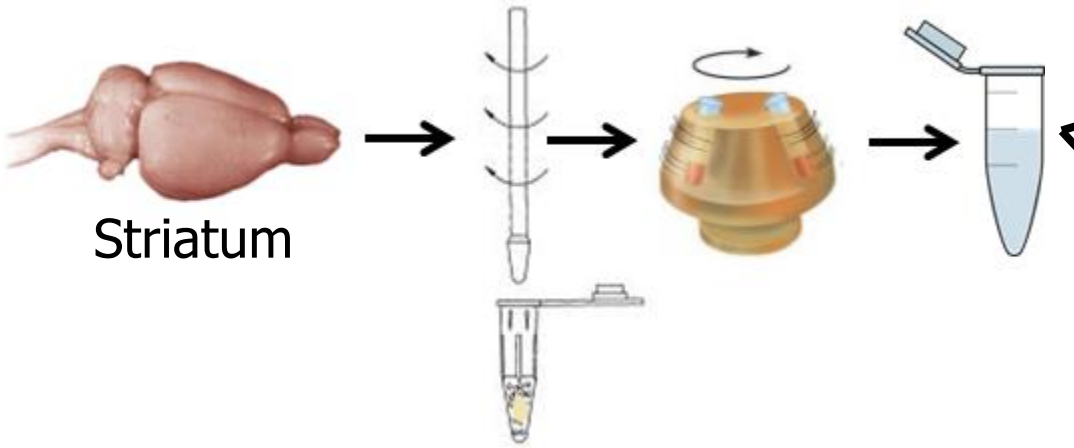
(ARAS et al., 2014)

Bradykinesia

Slow movements

Early postnatal exposure

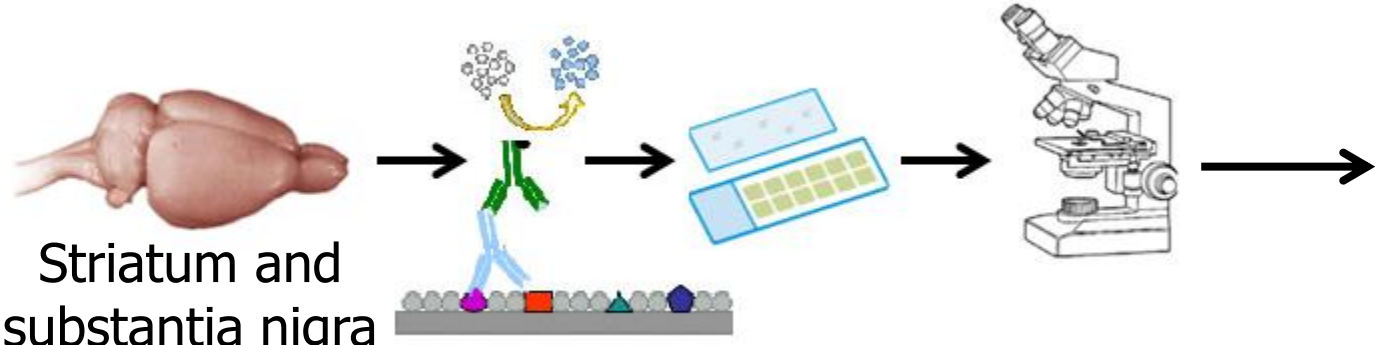
- Biochemical and immunohistochemistry



Striatum

Inflammatory parameters (IL-6, TNF-α)

Mitochondrial function (NADH dehygrogenase Complex II)



Striatum and substantia nigra

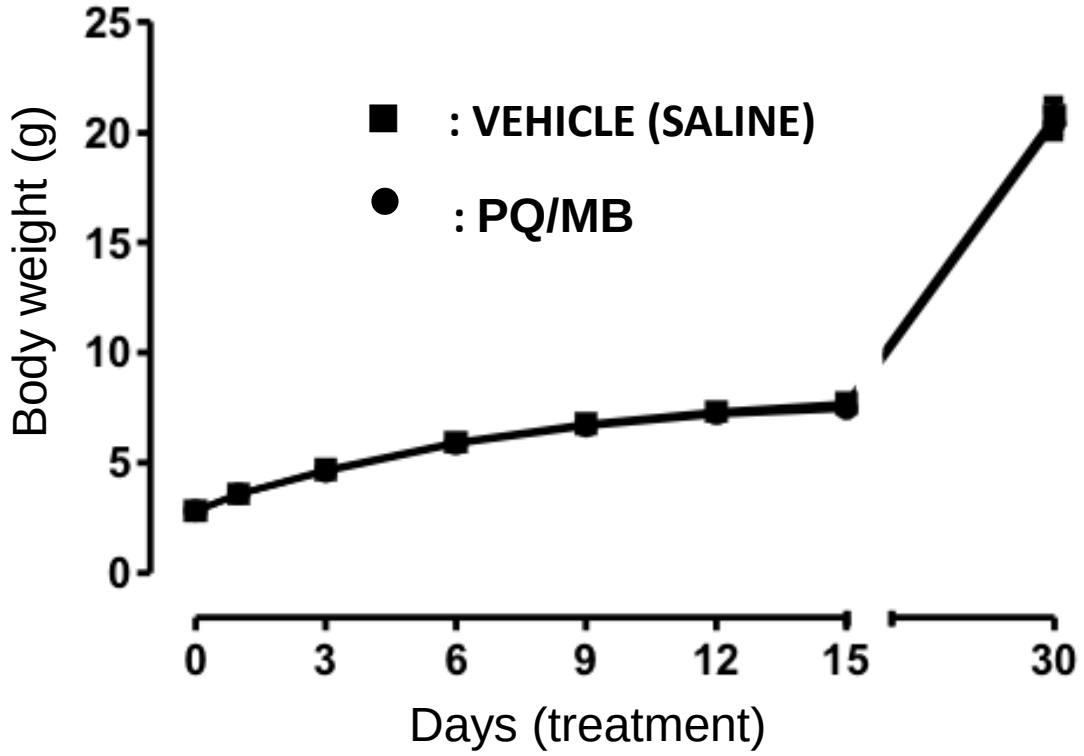
Marker of dopaminergic neurons: (Tyrosine hydroxylase – TH)

(Ellman, 1959; Misra & Fridovich, 1972; Aebi, 1974; Ohkawa et al., 1979; Wendel, 1981; Carlberg & Mannervik, 1985; Fischer et al., 1985; Cassina & Radi, 1996)

Early postnatal exposure

Body weight

Early postnatal exposure

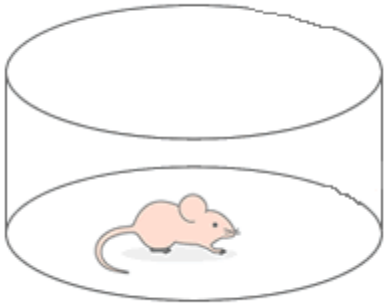


Colle et al., in preparation

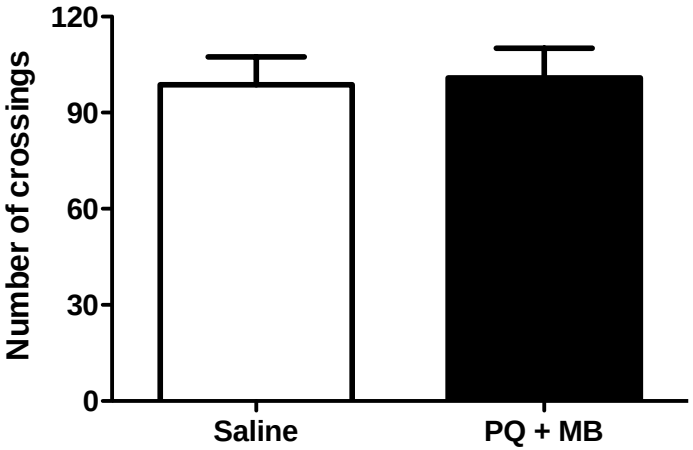
Open field task

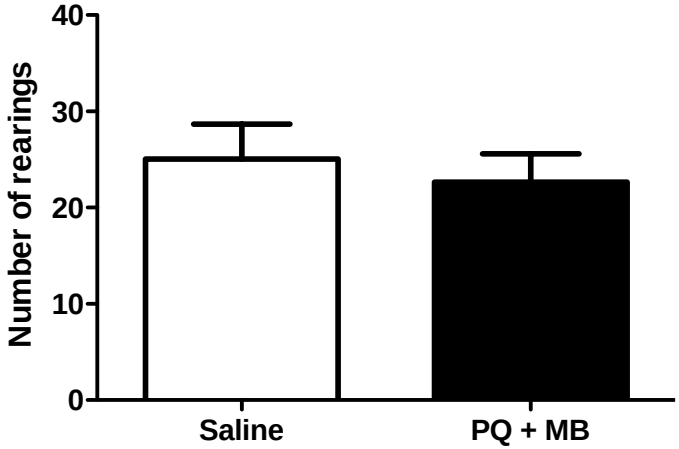
Early postnatal exposure

Crossings



Rearings

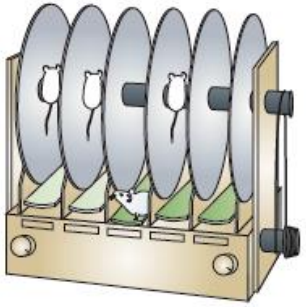




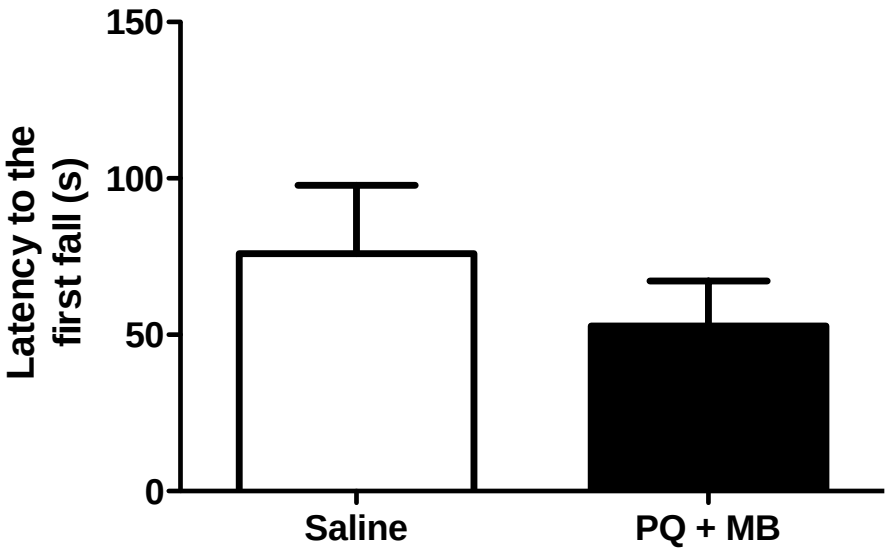
Colle et al., in preparation

Rotarod and pole test

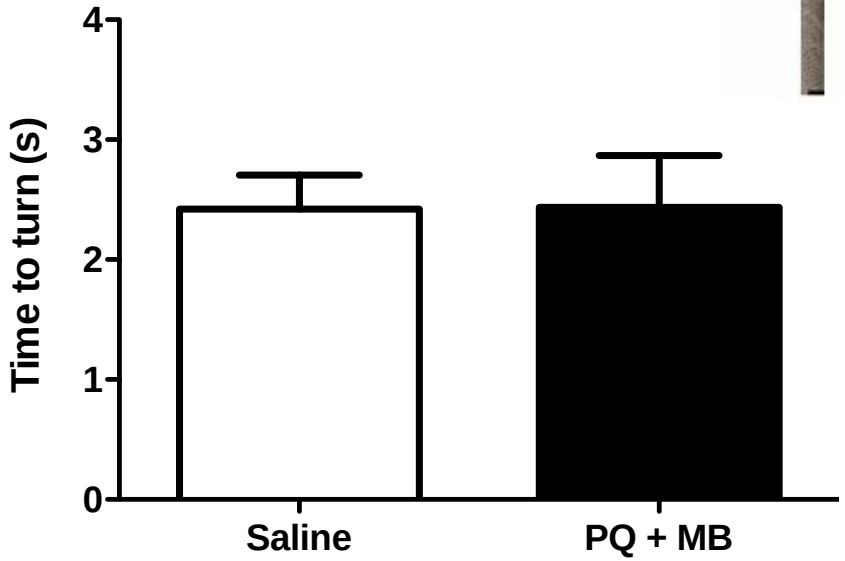
Early postnatal exposure



Rotarod: latency to fall



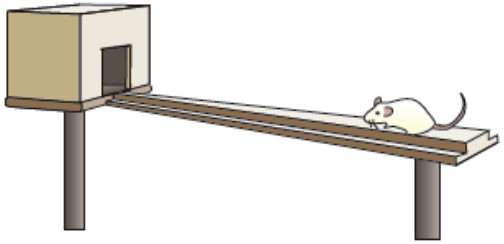
Pole test: latency to turn



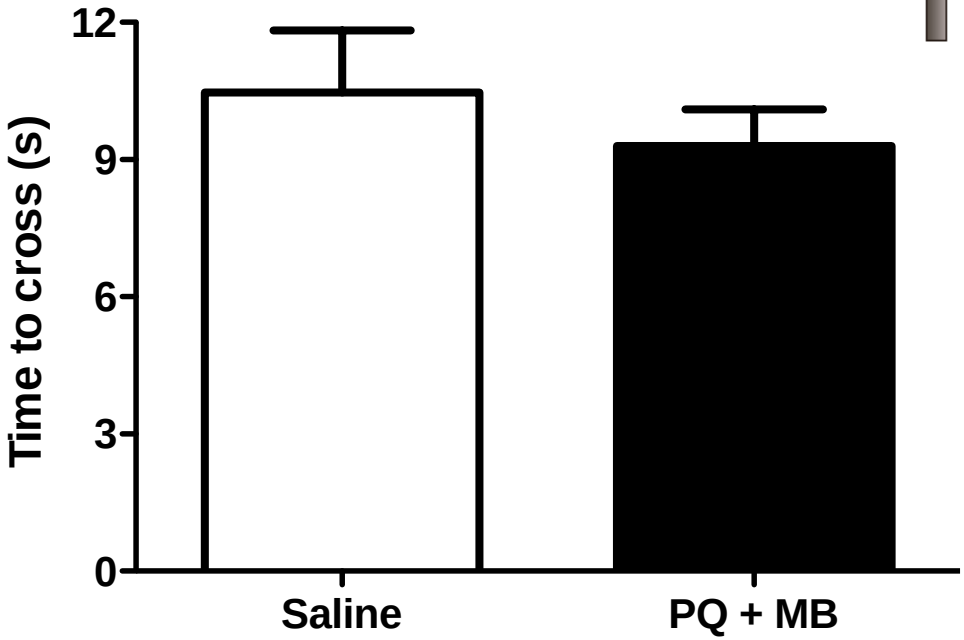
Colle et al., in preparation

Beam walking

Early postnatal exposure



Time to cross



Colle et al., in preparation

Striatal pro-inflammatory parameters

Early postnatal exposure

Groups	IL-6	TNF-α
Saline	43,16 ± 2,74	0,61 ± 0.37
PQ + MB	48,56 ± 6,33	1,32 ± 1,01

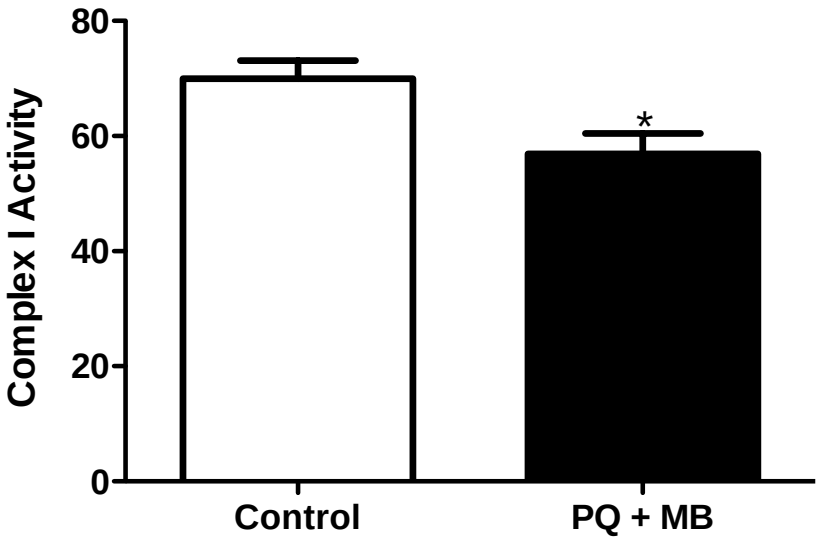
fg/mg protein

Colle et al., in preparation

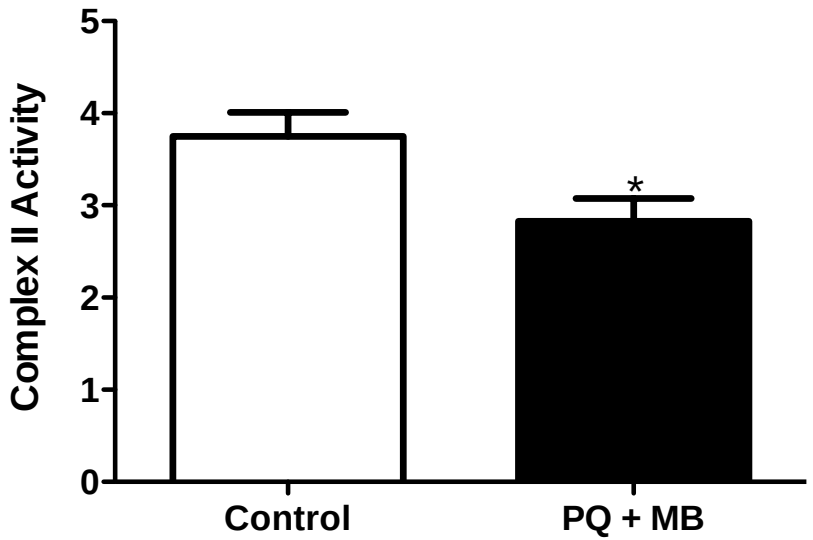
Striatal mitochondrial complexes I and II

Early postnatal exposure

Complex I



Complex II



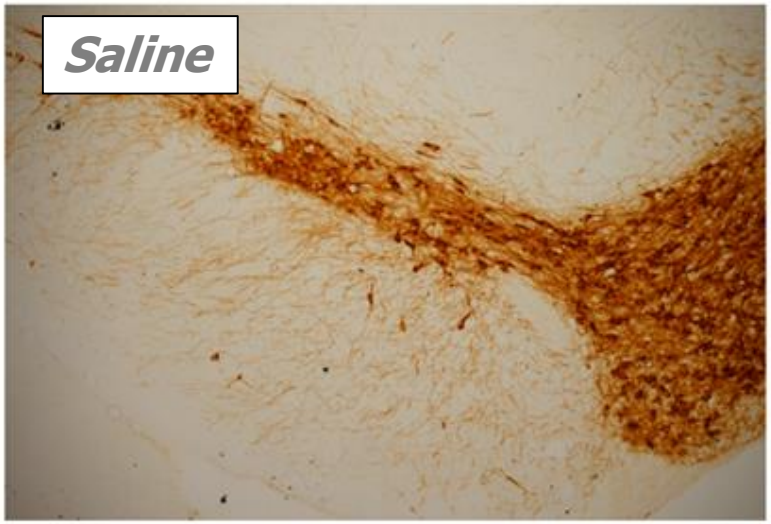
*$p < 0.05$

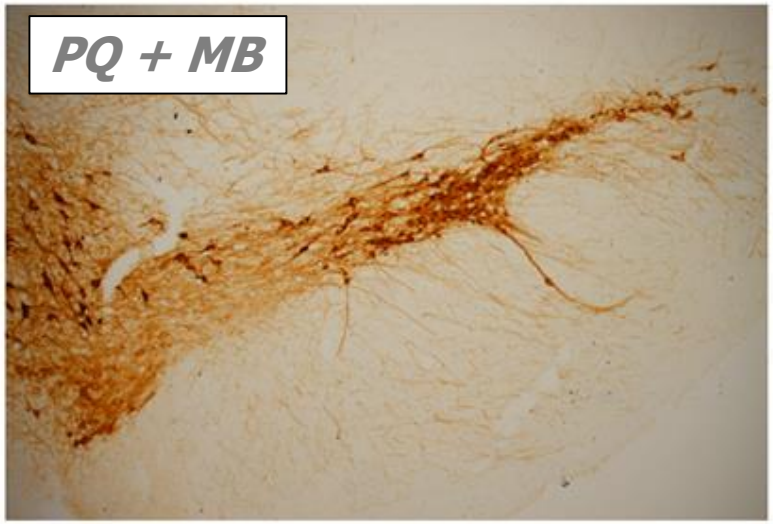
Colle et al., in preparation

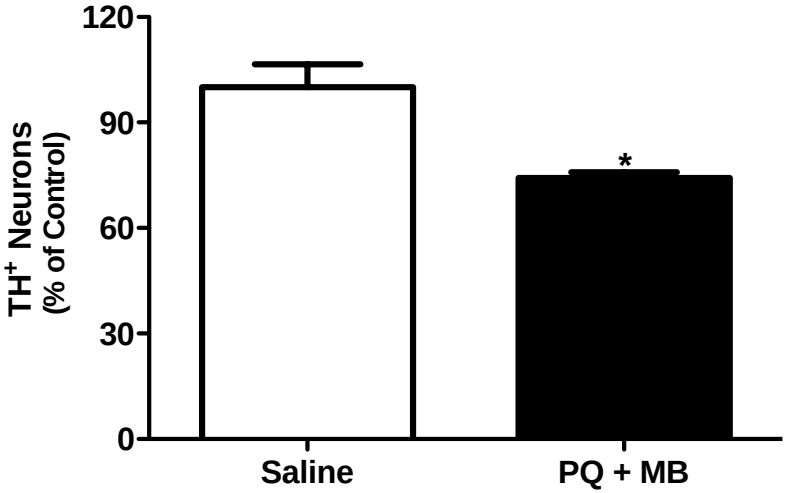
Substantia nigra

TH positive cells

Early postnatal exposure



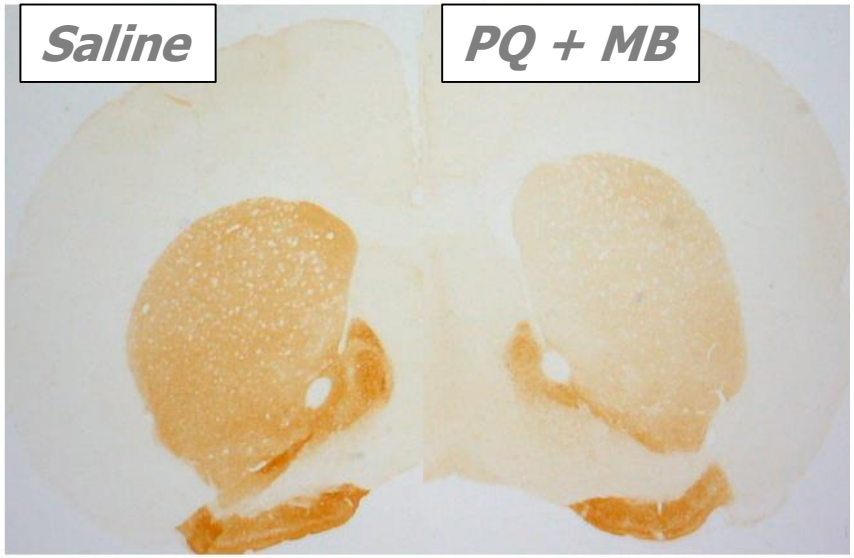


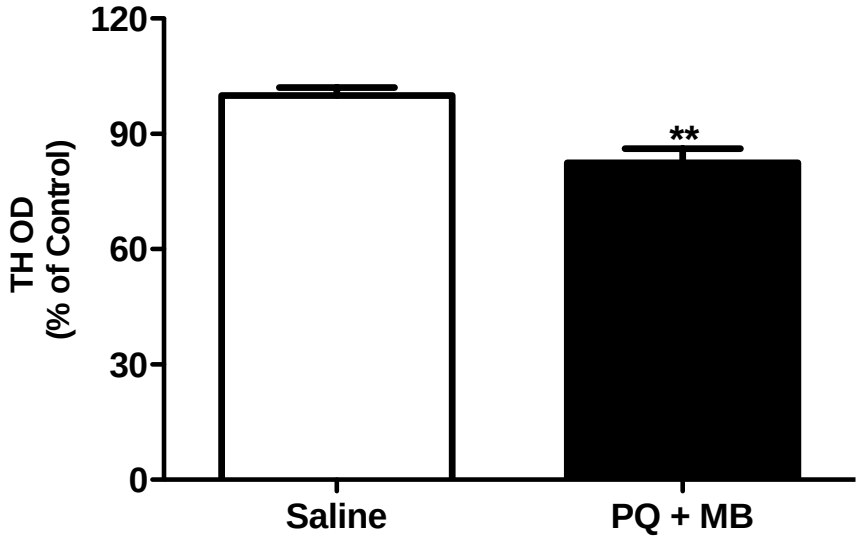


*$p < 0.05$

Striatum
TH staining

Early postnatal exposure



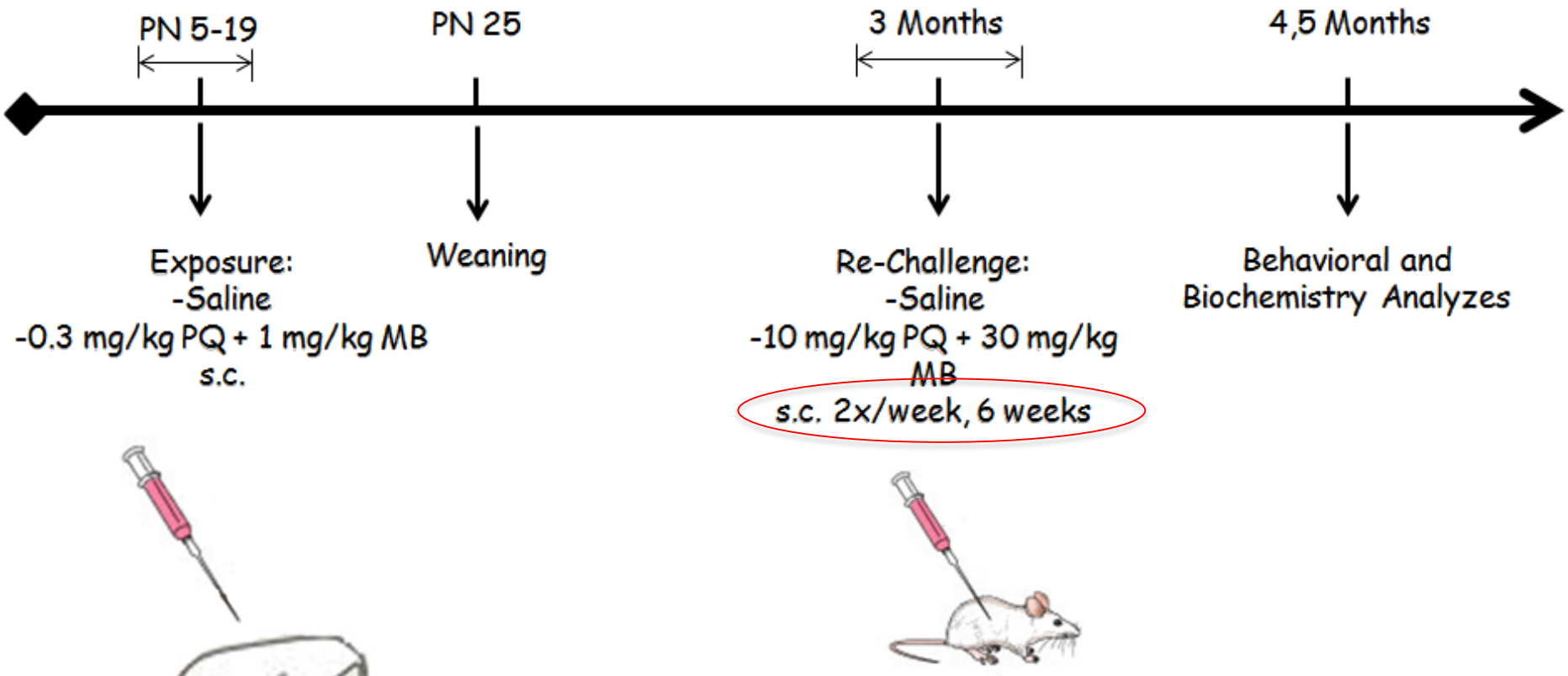


***p < 0.01*

Adult re-challenge

Adult re-challenge

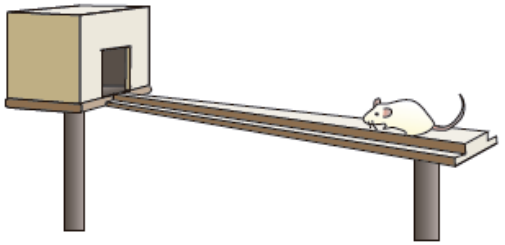
- **Males Swiss mice**: exposed to PQ + MB
- Protocol number: PP00765/CEUA/UFSC



THIRUCHELVAM et al., 2002, NeuroToxicol

- Behavioral analyses (motor-related parameters)

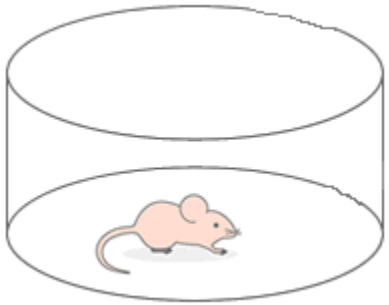
Adult re-challenge



Beam walking

(KHARIV et al., 2013)

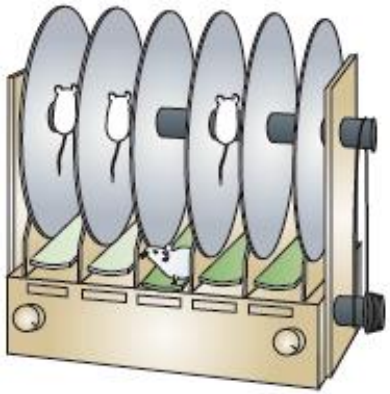
Coordination and balance



Open field

(SANTOS et al., 2012)

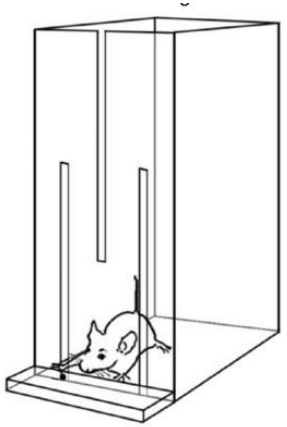
Locomotor and exploratory activities



Rotarod

(KHARIV et al., 2013)

Motor performance



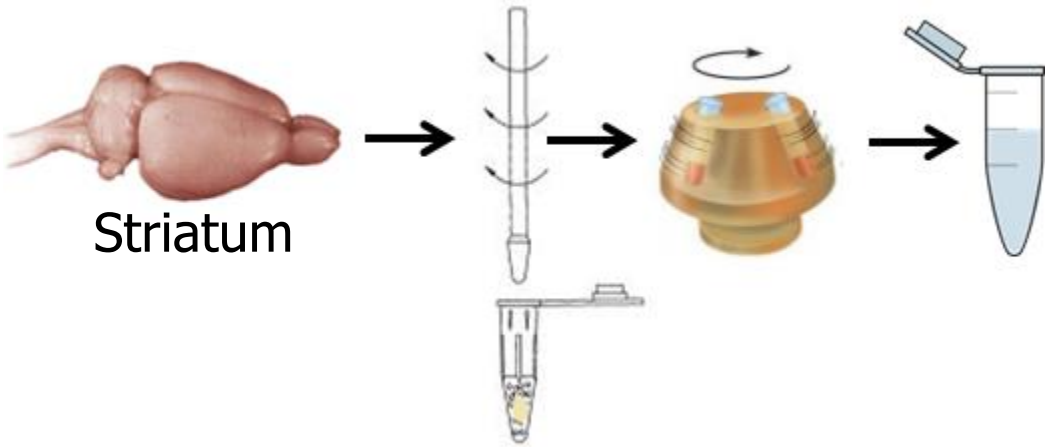
Single pellet

(CHEN et al., 2014)

Fine coordination

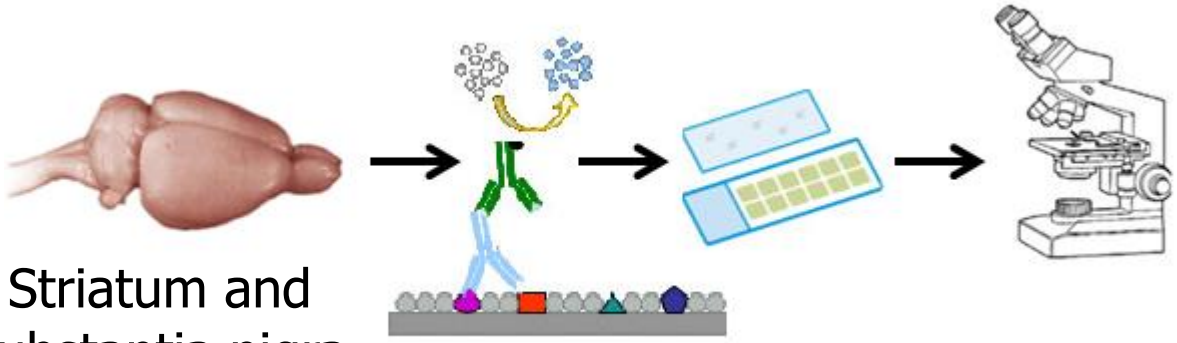
Adult re-challenge

- Biochemical and immunohistochemistry



Striatum

Mitochondrial function
(NADH dehygrogenase Complex II)



Striatum and substantia nigra

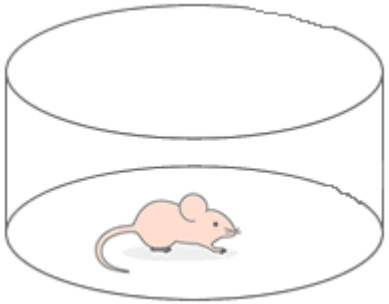
Marker of dopaminergic neurons:
(Tyrosine hydroxylase – TH)

(Fischer et al., 1985; Cassina & Radi, 1996)

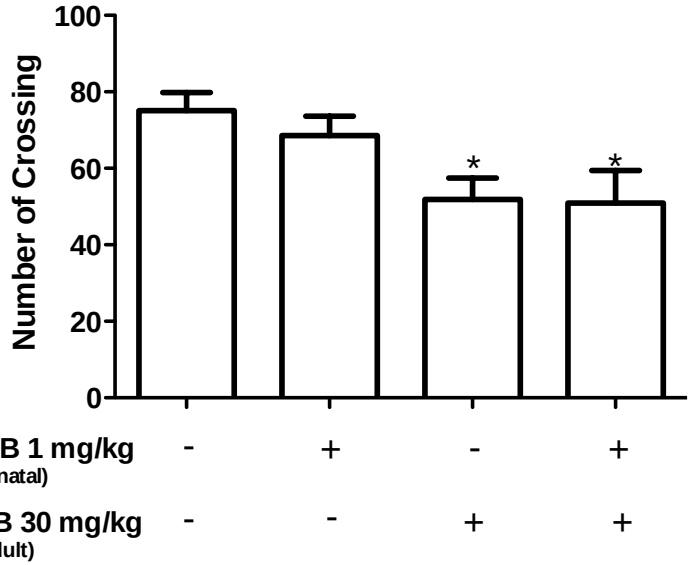
Adult re-challenge

Open field task

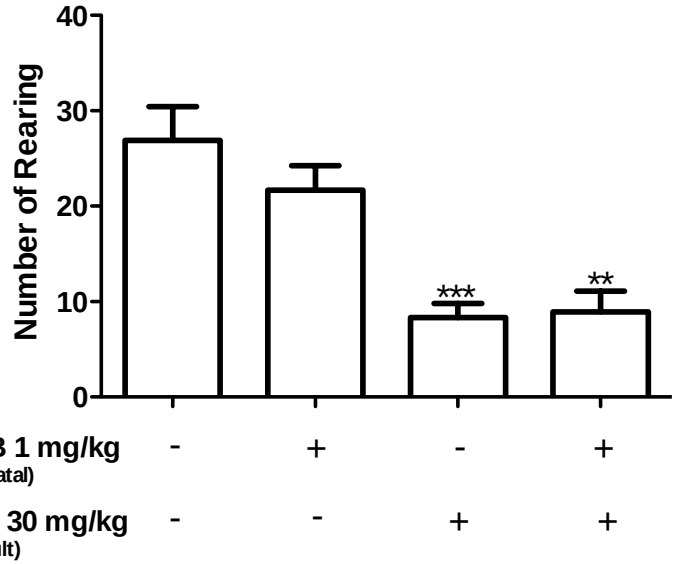
Adult re-challenge



Crossings



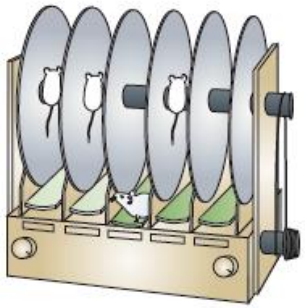
Rearings



**p < 0.05; **p < 0.01; ***p < 0.001*

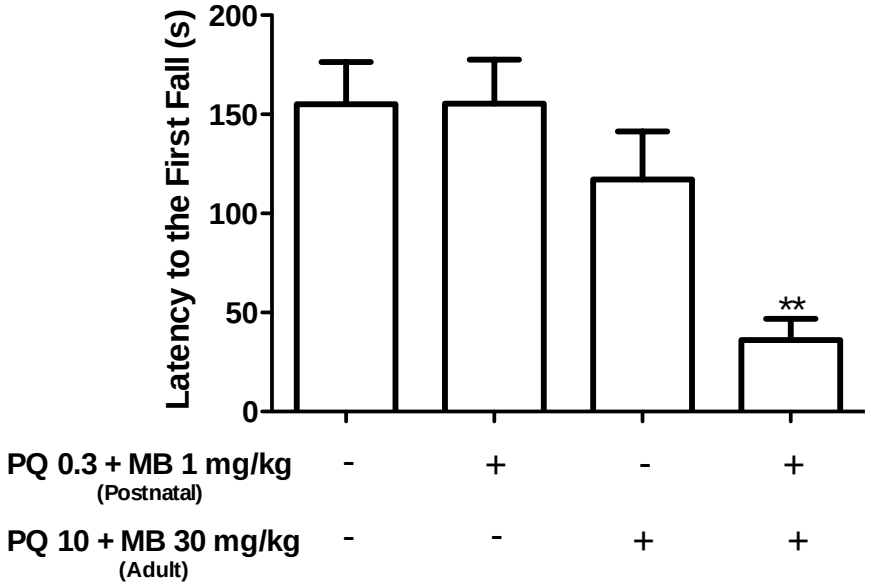
Colle et al., in preparation

Rotarod

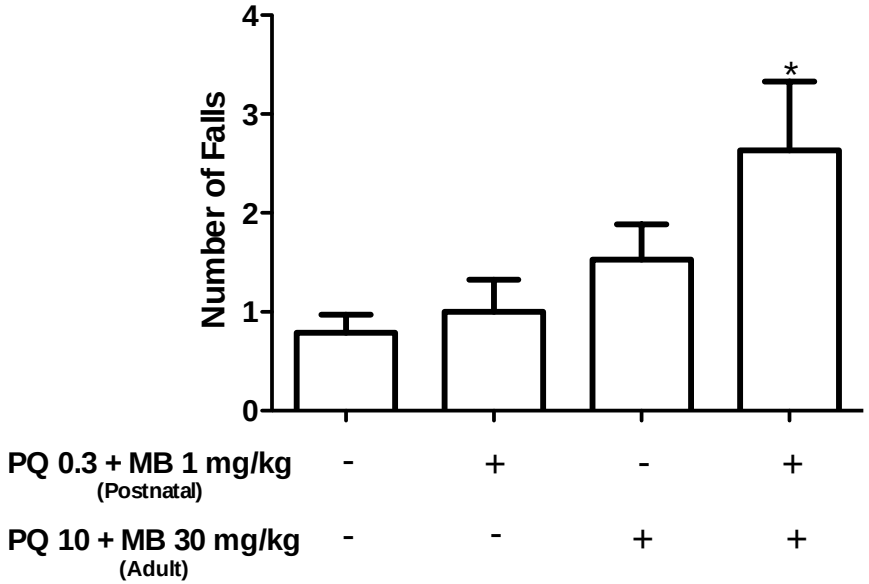


Adult re-challenge

Latency to fall



Number of falls

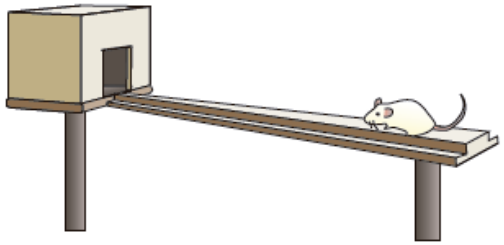


**p < 0.05; **p < 0.1*

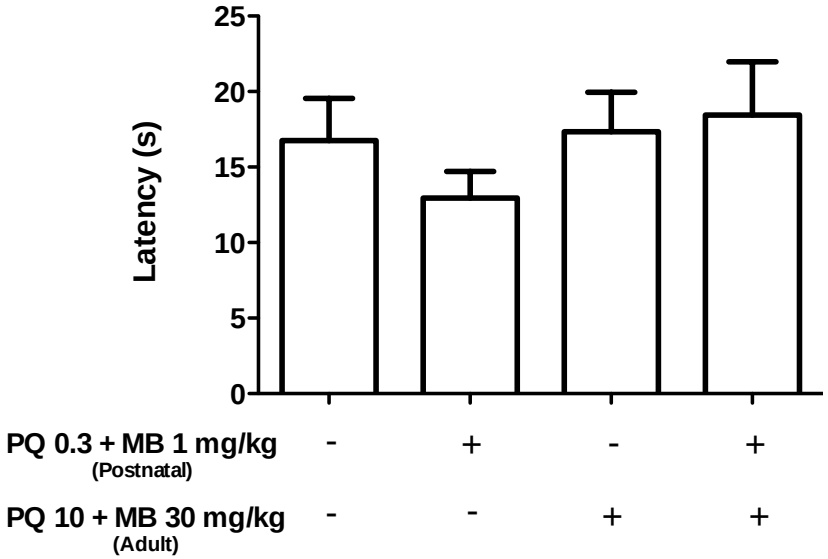
Colle et al., in preparation

Beam walking

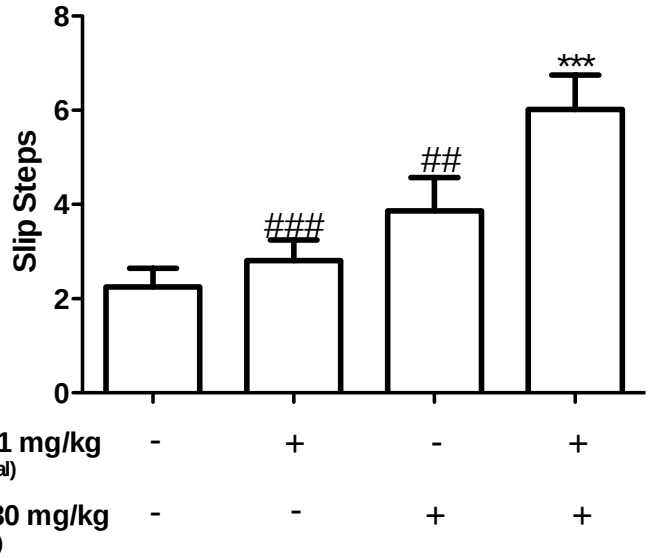
Adult re-challenge



Latency to fall



Slip steps

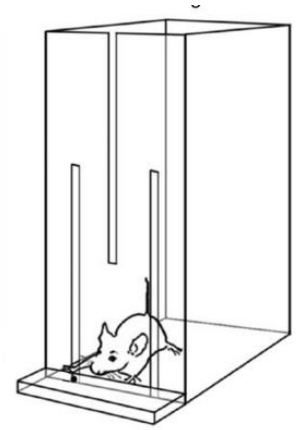


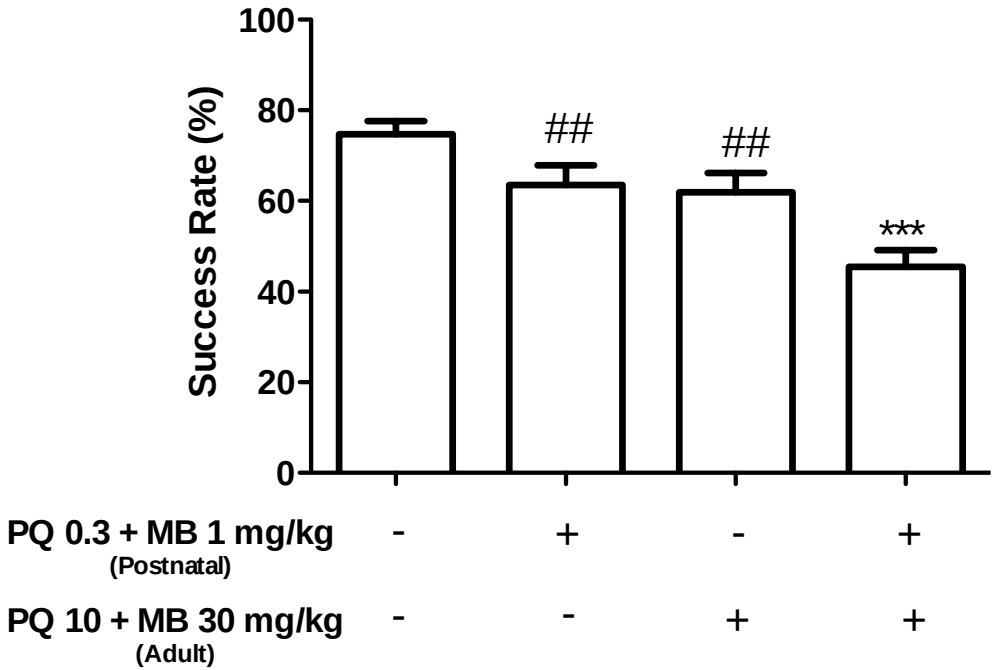
****p < 0.001 compared to control*
##p < 0.01 and ###p < 0.001 compared to the group exposed at both periods

Colle et al., in preparation

Single pellet

Early postnatal exposure



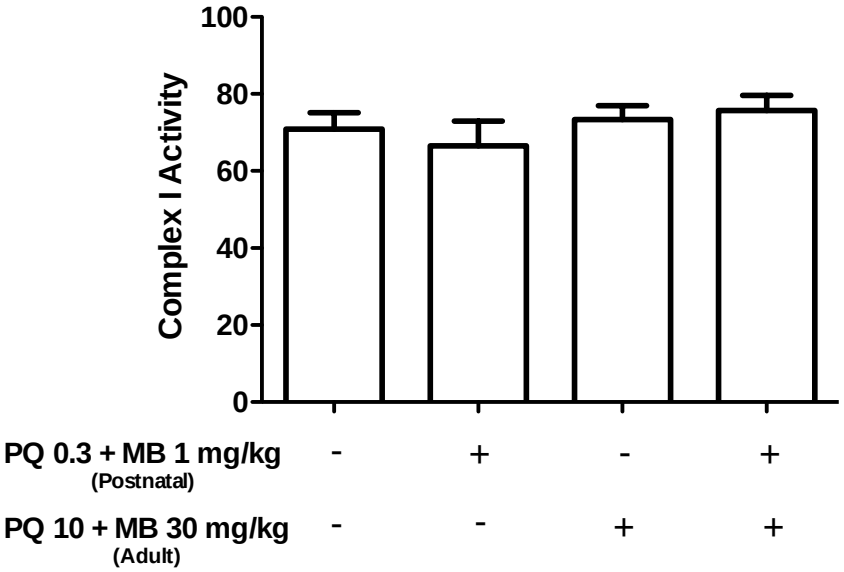


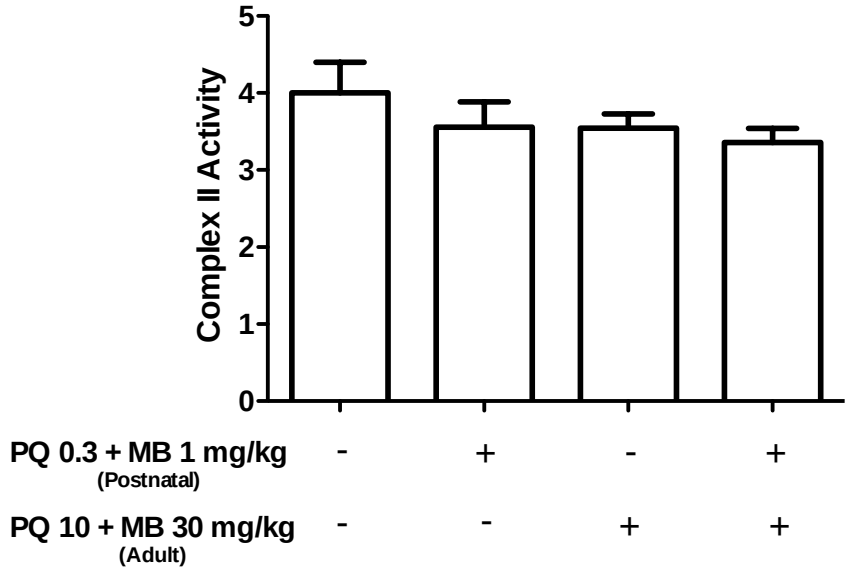
***p < 0.001 compared to control*
##p < 0.01 compared to the group exposed at both periods

Colle et al., in preparation

Striatal mitochondrial complexes I and II

Adult re-challenge

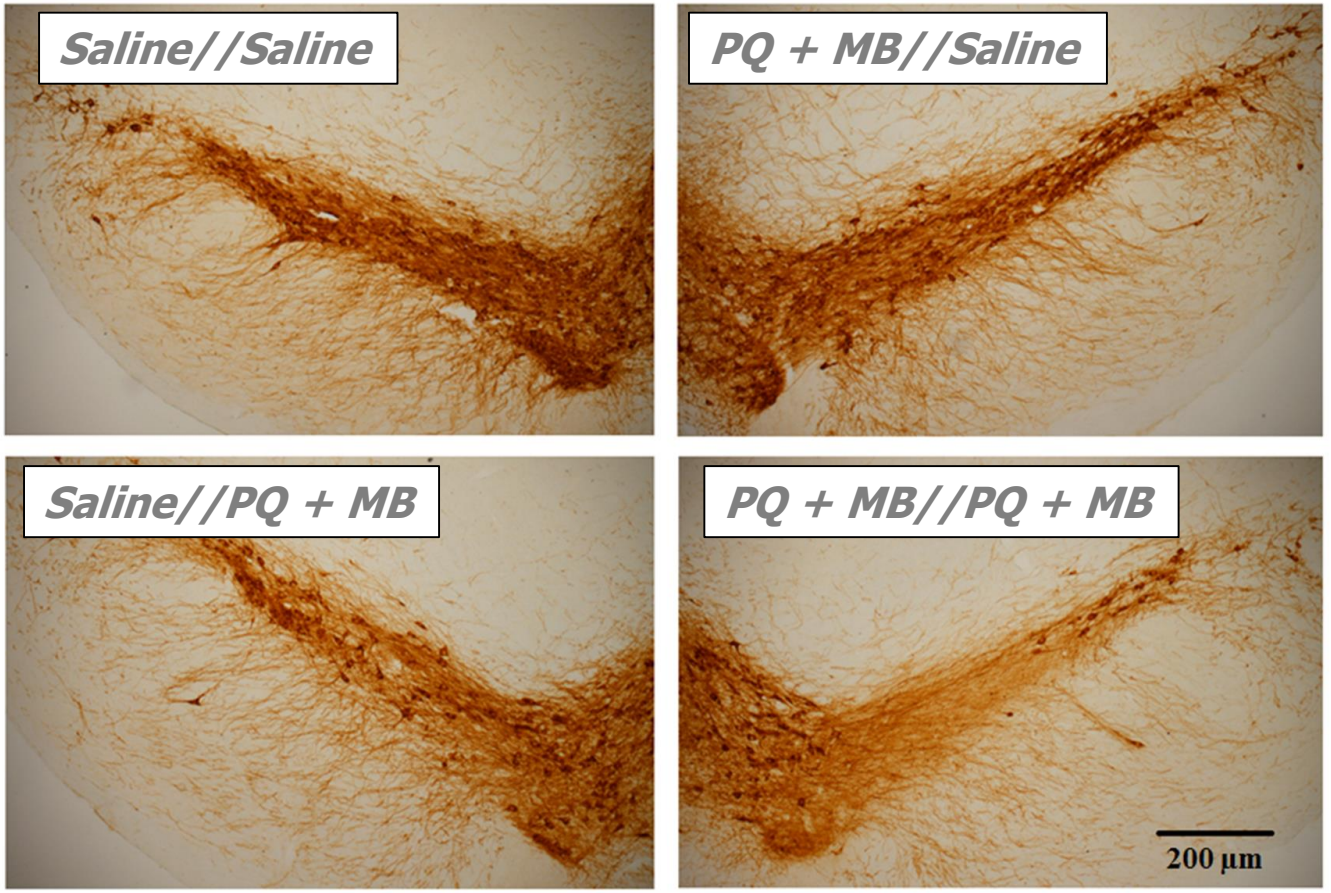




Colle et al., in preparation

Adult re-challenge

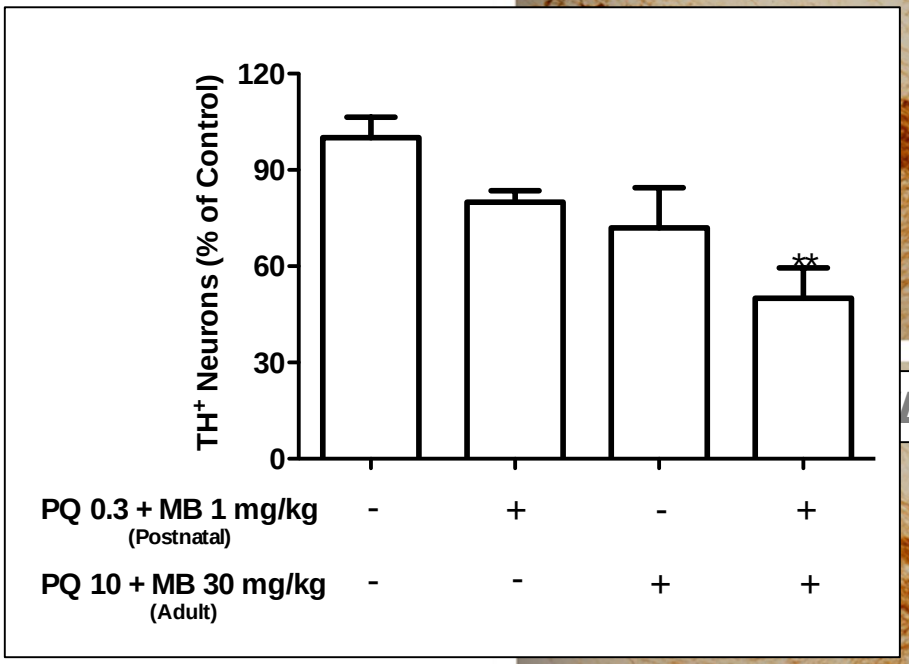
Substantia nigra
TH positive cells

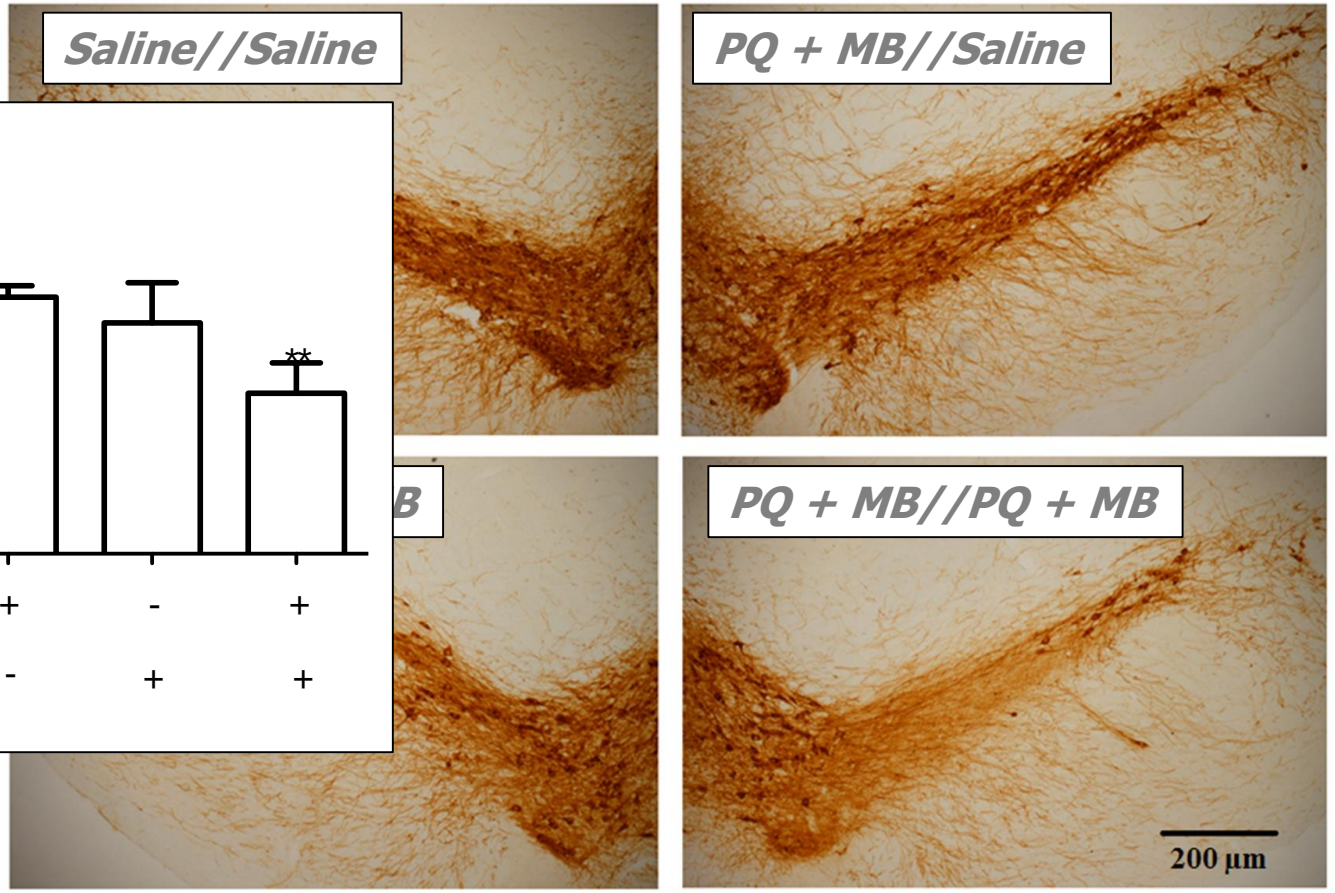


Colle et al., in preparation

Adult re-challenge

Substantia nigra
TH positive cells



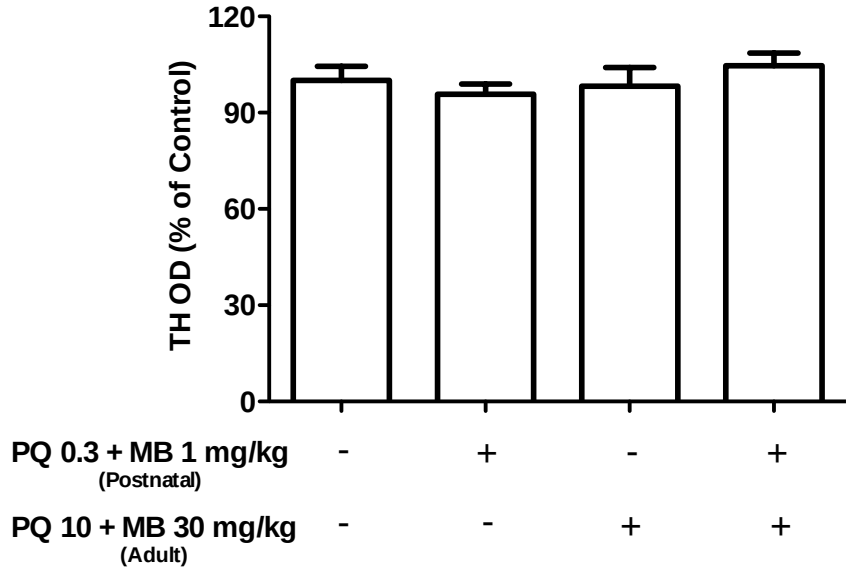


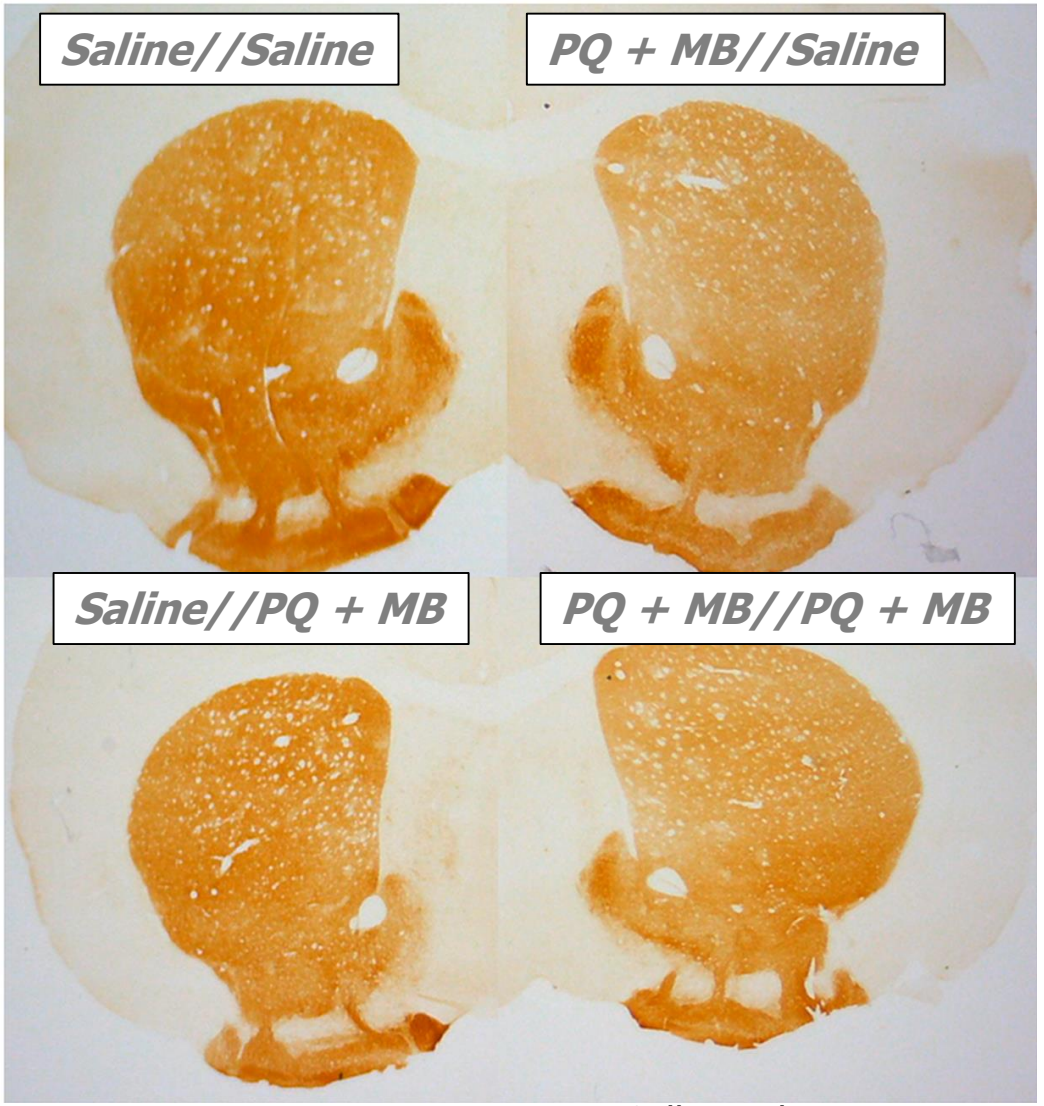
***p < 0.01 compared to control*

Colle et al., in preparation

Adult re-challenge

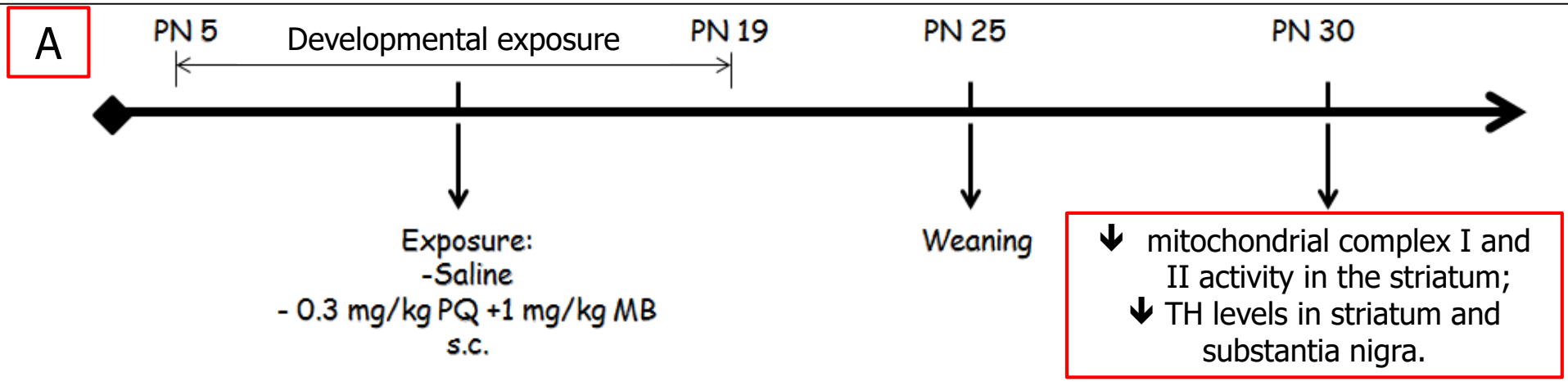
Striatum
TH staining



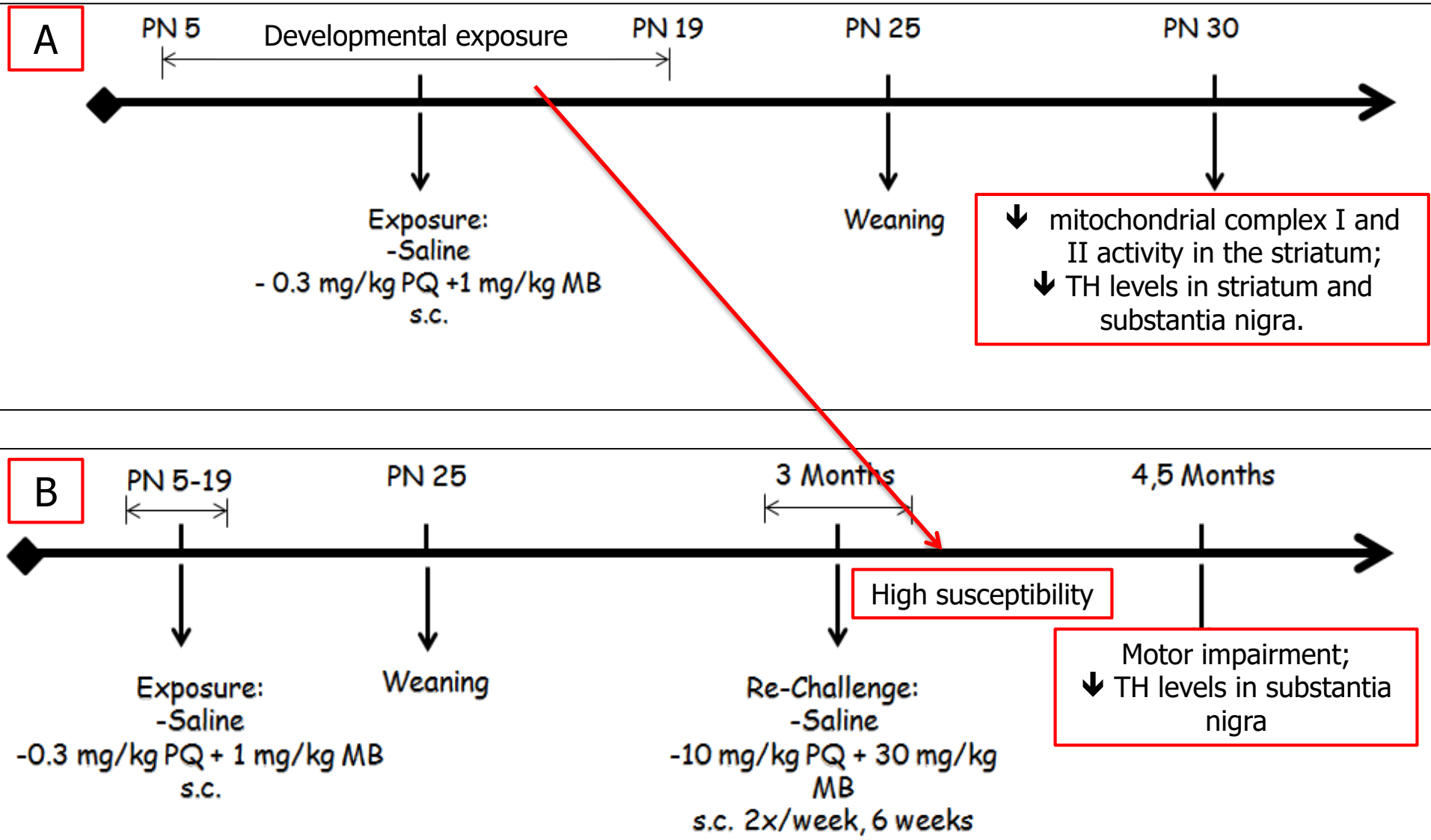


Colle et al., in preparation

Conclusions



Conclusions



Thanks!

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FAPESC

CAPES

Thanks for your attention!

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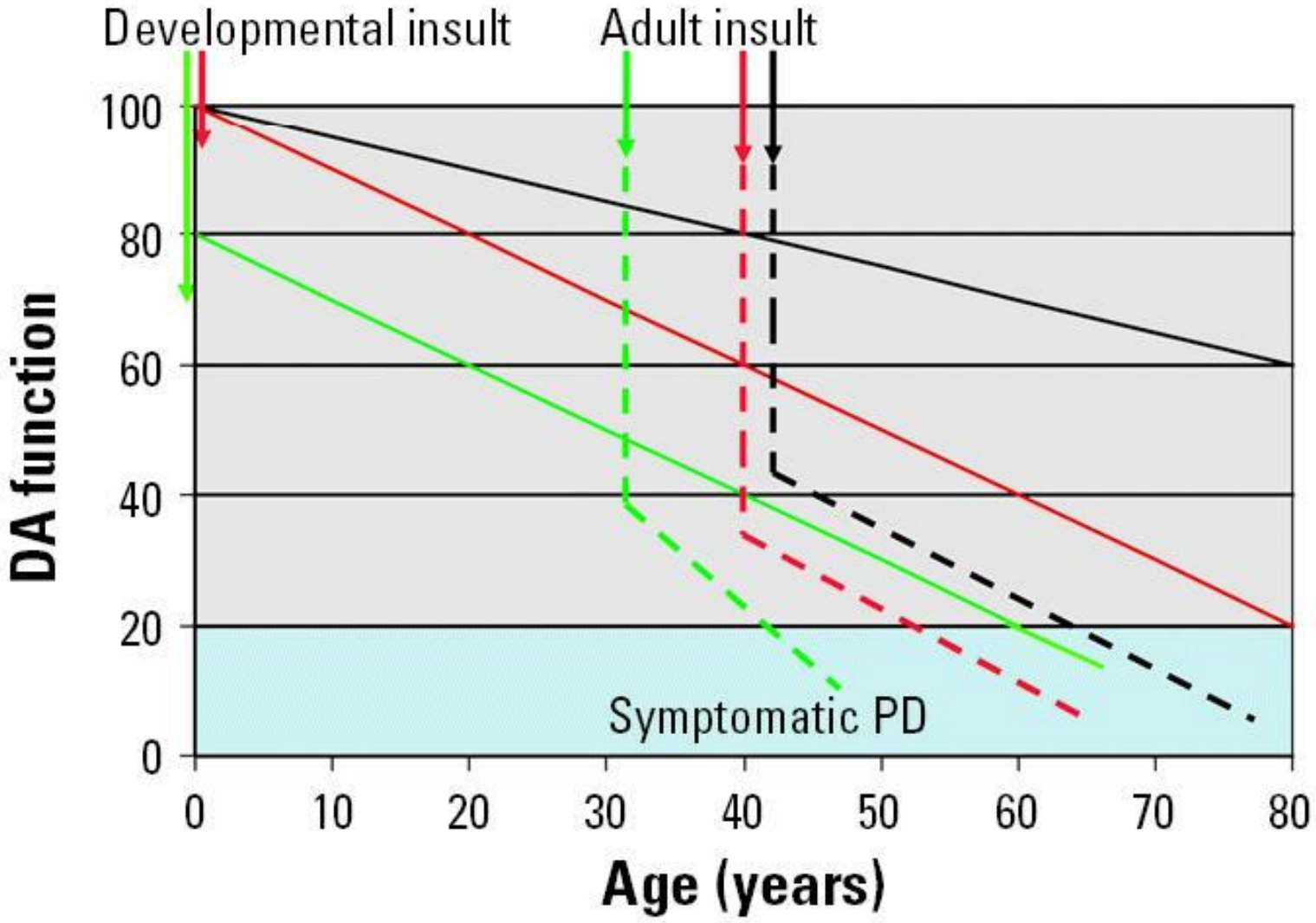
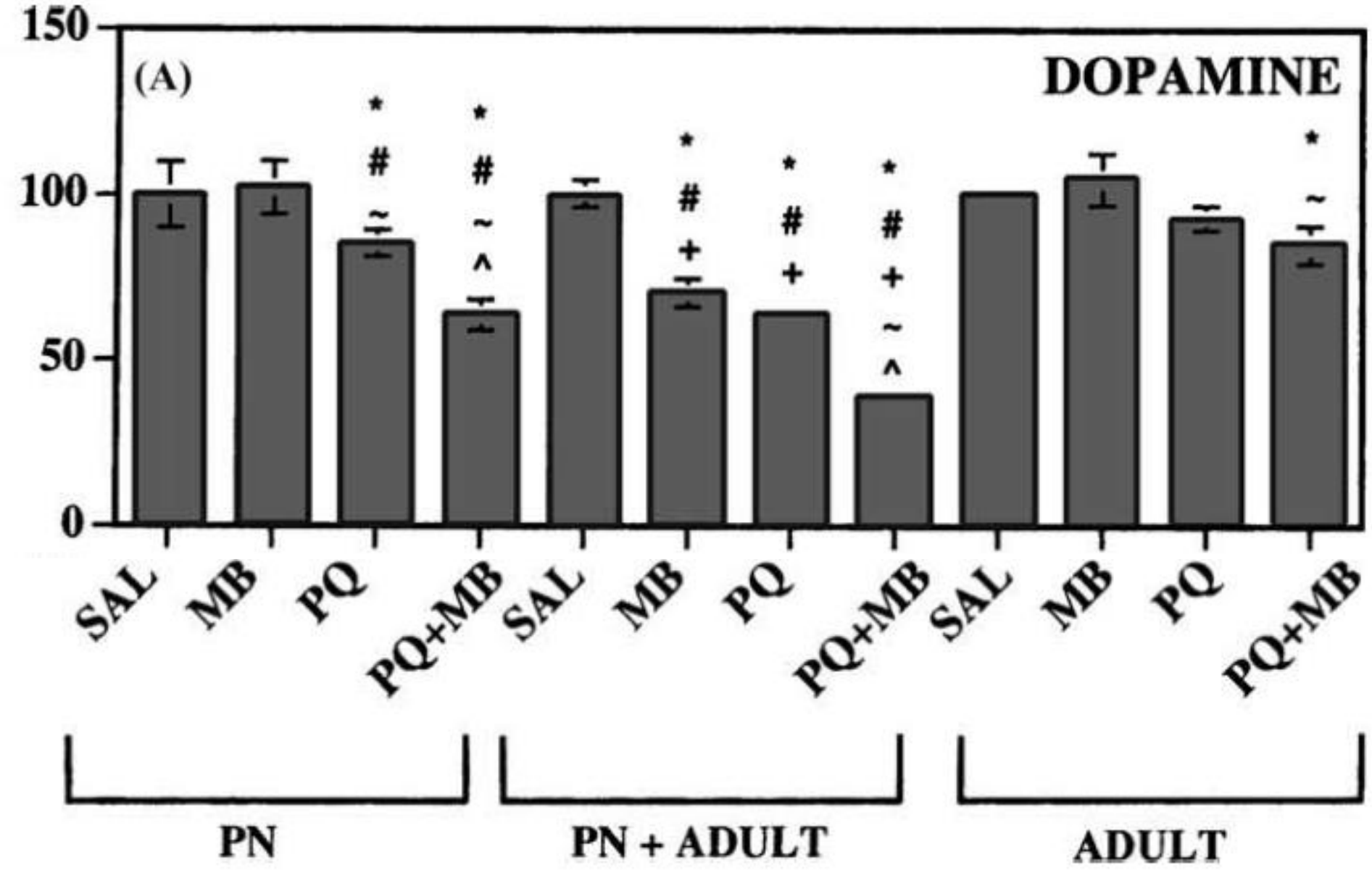
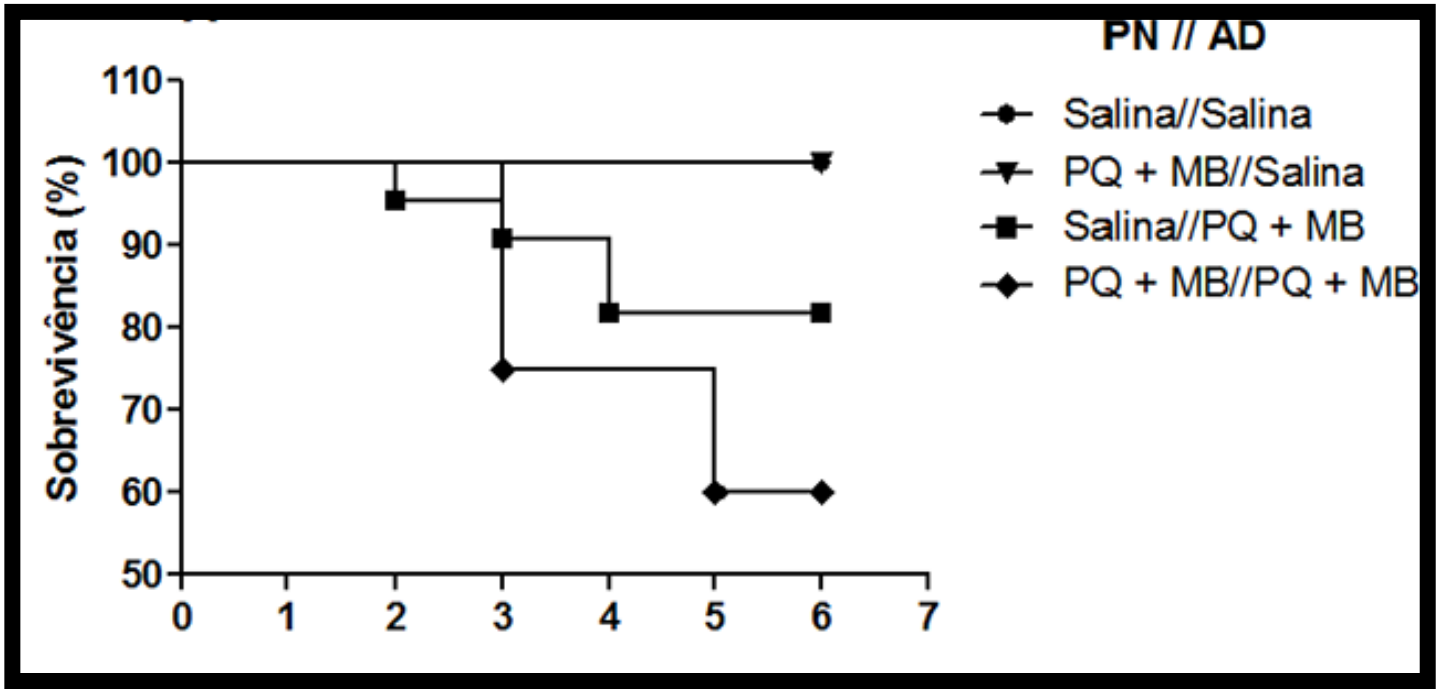
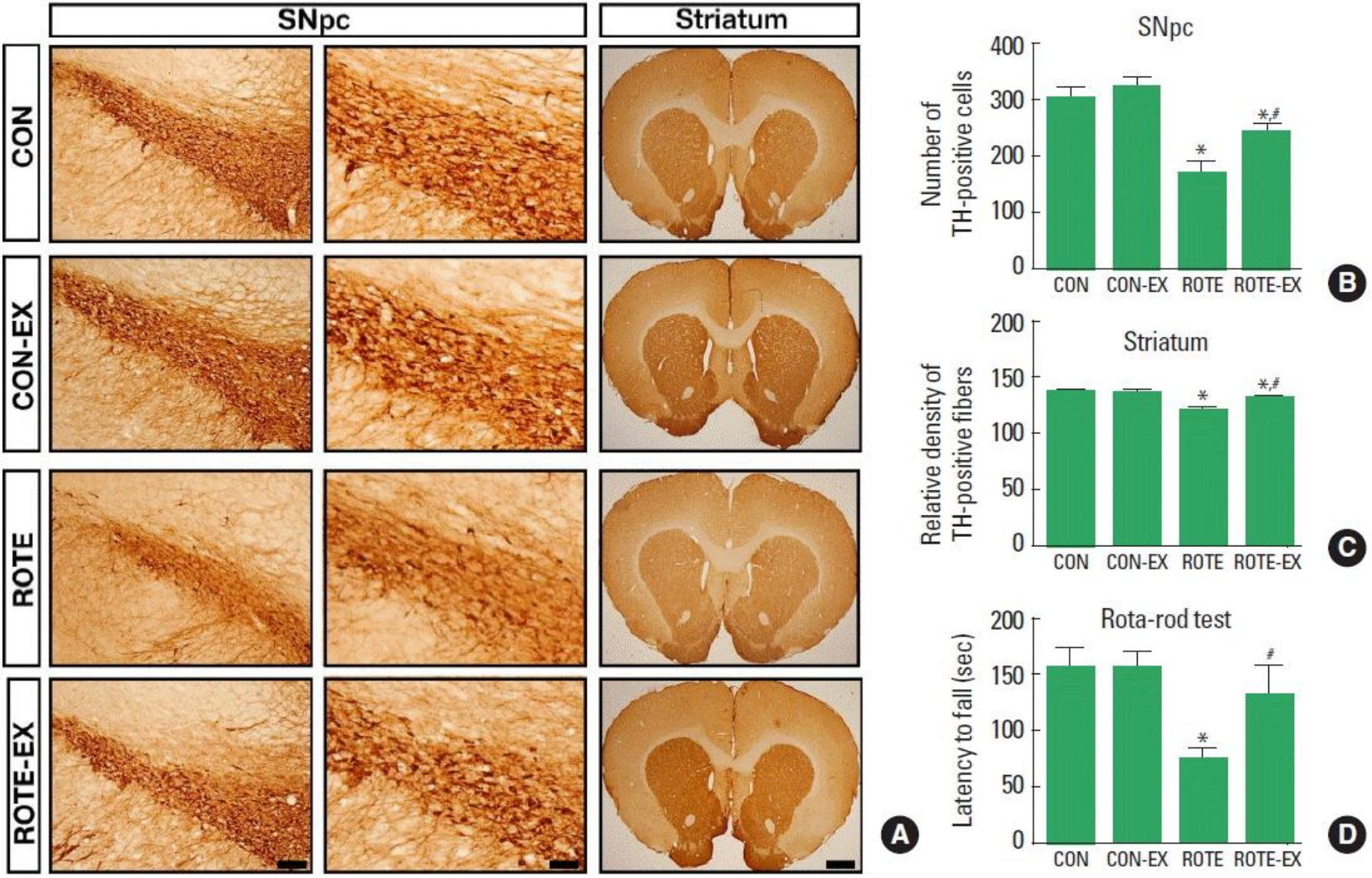
Developmental exposure



Cory-Slechta et al., 2005, EHP


150
100
50
0
(A)
DOPAMINE
SAL
MB
PQ
PQ+MB
SAL
MB
PQ
PQ+MB
SAL
MB
PQ
PQ+MB
PN
PN + ADULT
ADULT


PN // AD
Salina//Salina
PQ + MB//Salina
Salina//PQ + MB
PQ + MB//PQ + MB
Sobrevivência (%)
110
100
90
80
70
60
50
0
1
2
3
4
5
6
7


SNpc
Striatum
CON
CON-EX
ROTE
ROTE-EX
A
SNpc
Number of TH-positive cells
400
300
200
100
0
*
*,#
CON
CON-EX
ROTE
ROTE-EX
B
Striatum
Relative density of TH-positive fibers
200
150
100
50
0
*
*,#
CON
CON-EX
ROTE
ROTE-EX
C
Rota-rod test
Latency to fall (sec)
200
150
100
50
0
*
#
CON
CON-EX
ROTE
ROTE-EX
D

Brain Res. 1996 Feb 19;709(2):319-25.

Dopaminergic sprouting in the rat striatum after partial lesion of the substantia nigra.

Blanchard V[1], Anglade P, Dziewczapolski G, Savasta M, Agid Y, Raisman-Vozari R.

Author information

Abstract

The capacity of the dopaminergic nerve system to reinnervate the denervated adult striatum was analyzed in a model of partial 6-hydroxydopamine-induced unilateral lesion of rat substantia nigra pars compacta. Sprouting of dopaminergic fibers entering the ventrolateral part of the striatum from a narrow zone of the external capsule was detected on the lesioned side 4 and 7 months, but not 10 days, after lesioning. Ultrastructural examination of the zone of sprouting revealed hypertrophic dopaminergic fibers and growth-cone-like structures, confirming the existence of an ongoing process of spontaneous regrowth of dopaminergic fibers. The identification of the factors involved in the regrowth of dopaminergic fibers may help to orientate molecular research into new treatments for Parkinson's disease.

Brain Res. 1995 Jan 30;670(2):197-204.

Sprouting of dopaminergic fibers from spared mesencephalic dopamine neurons in the unilateral partial lesioned rat.

Hansen JT[1], Sakai K, Greenamyre JT, Moran S.

Author information

Abstract

A unilateral partially lesioned rat model of Parkinson's disease was developed following selective lesioning of the dopamine neurons of the substantia nigra pars compacta by stereotactic injection of the neurotoxin 6-hydroxydopamine. In this animal model the dopamine neurons of the ventral tegmental area and medial substantia nigra are spared. The neuronal loss in such partial lesioned models mimics more closely that seen in human mid-stage parkinsonism. Cografts of adrenal medullary cells and sciatic nerve to the partially lesioned striatum induced a sprouting response in grafted animals that was confirmed by immunocytochemical staining with antibodies to tyrosine hydroxylase (TH) and by quantification of the high affinity dopamine uptake complex using [3H]GBR 12935 binding. Enhanced TH fiber immunostaining was evident even in the presence of poor cograft survival. The origin of the TH-like immunostained fibers in the striatum was determined using Lucifer yellow retrograde axonal transport. Following discrete tracer injections into the striatum adjacent to a cograft, neurons in the medial substantia nigra and ventral tegmental area (areas A9 and A10, respectively) were labeled with Lucifer yellow. These labelled neurons displayed a morphology characteristic of dopamine neurons and, in double-labelling experiments, also immunostained for TH. These results support the utility of unilateral partially lesioned rat models of Parkinson's disease for studies investigating a host sprouting or upregulation response and confirm that the immunostained striatal fibers originate from spared dopamine neurons in the ventromedial midbrain.