In vivo efficacy testing of nanodrugs for Parkinson's disease treatment

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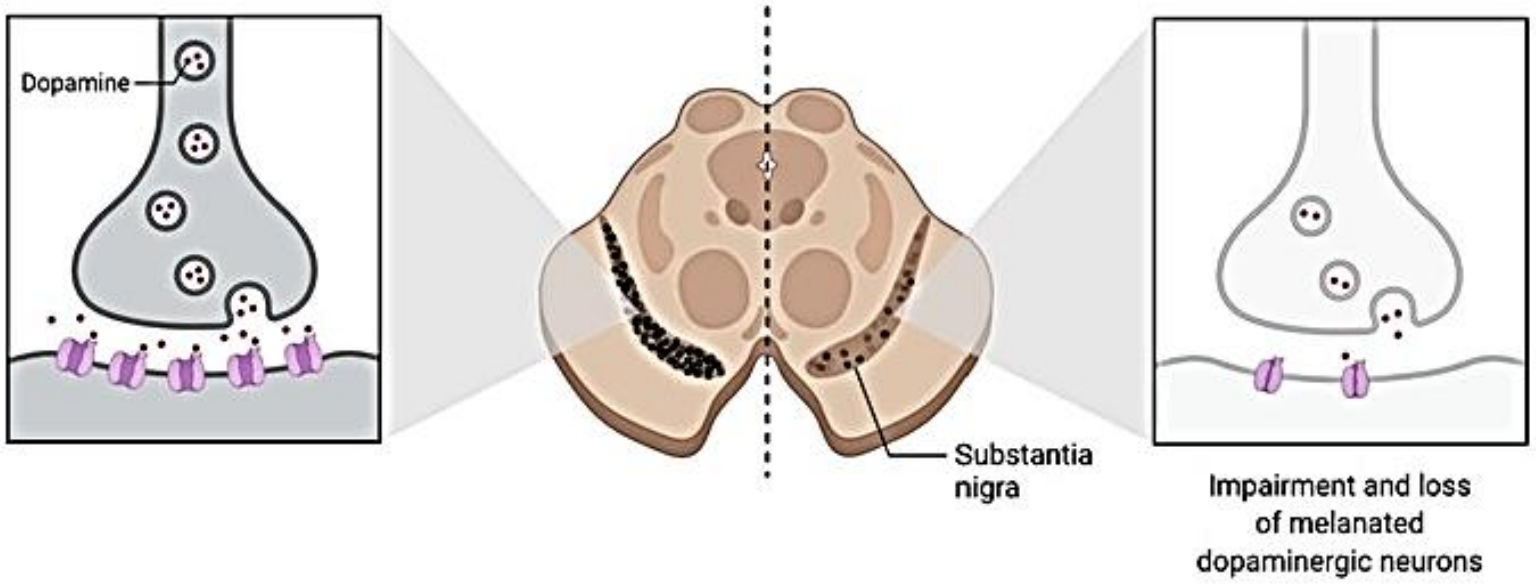
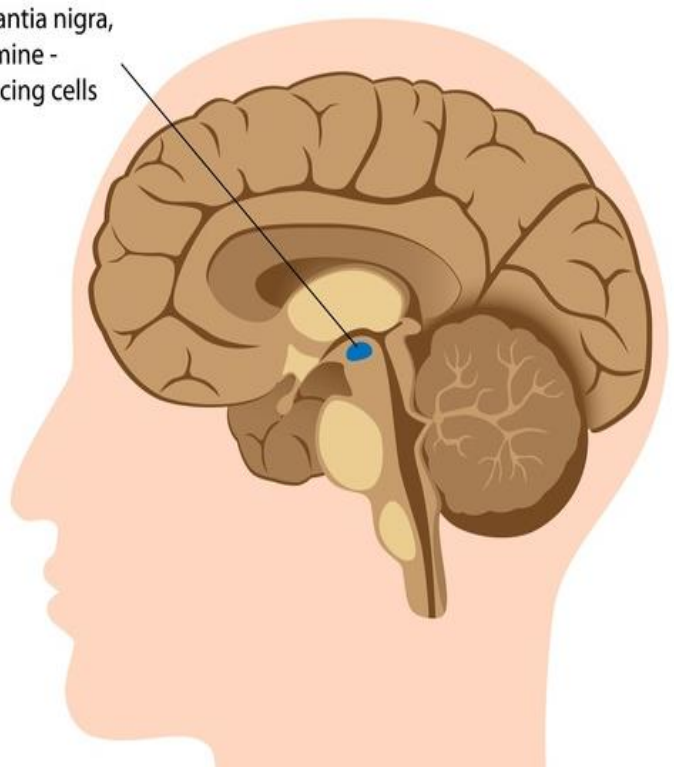
Parkinson's disease (PD)

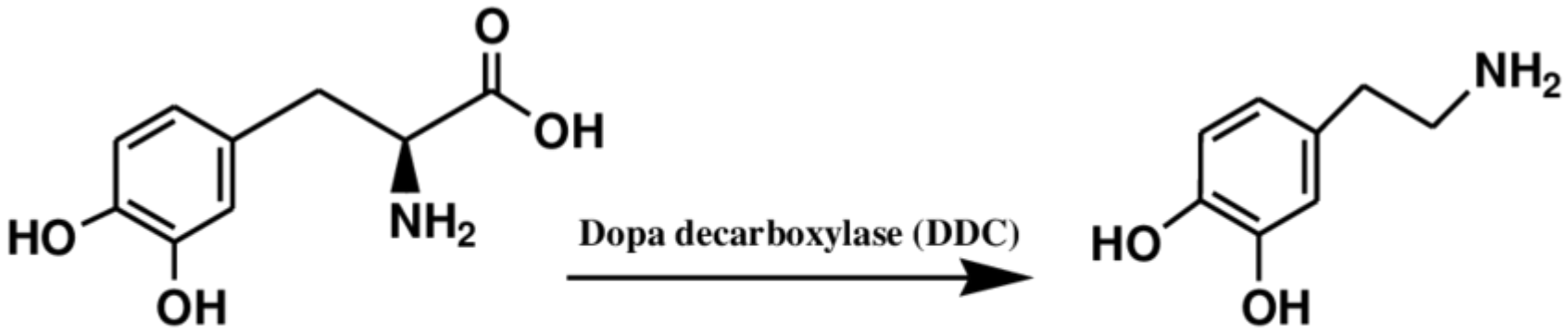
- massive depletion of dopamine
- motor symptoms - tremor, muscle stiffness
- neuropsychiatric and cognitive symptoms





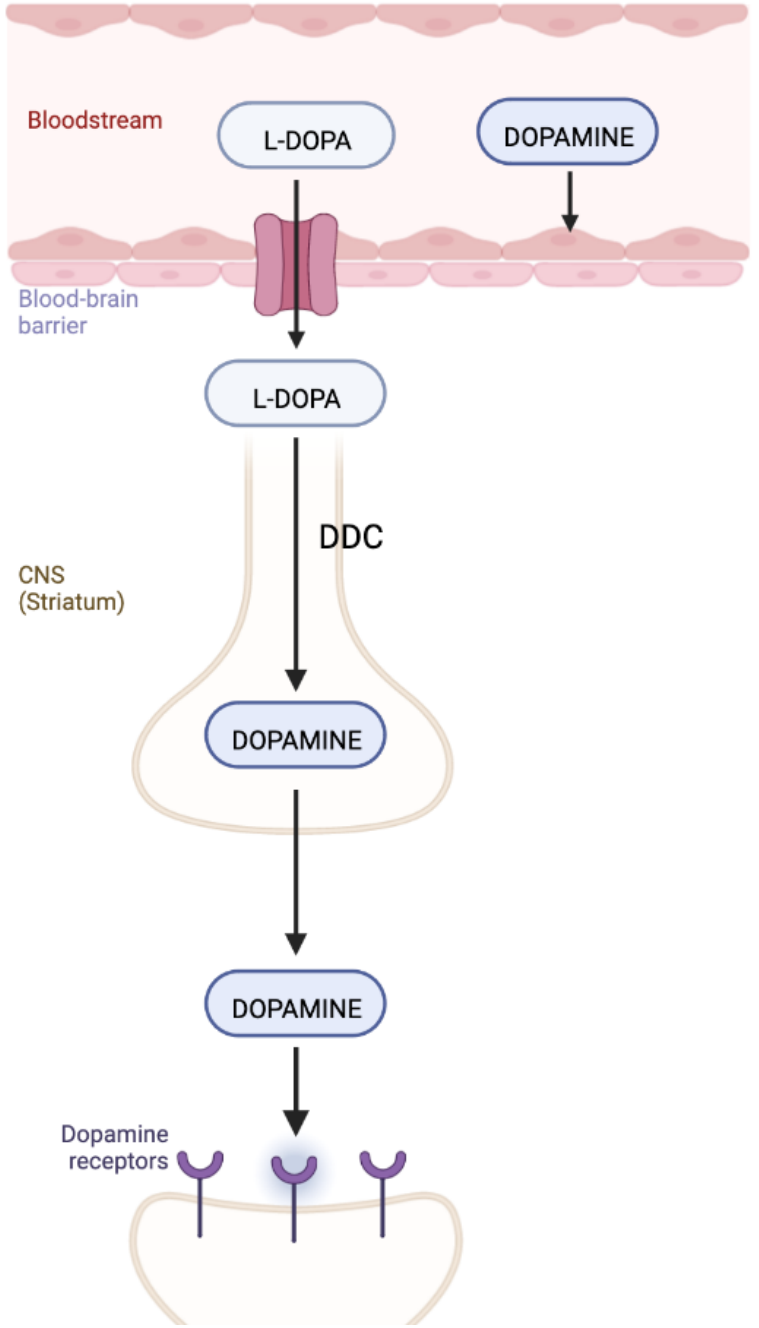
Conventional therapy for PD

- Levodopa (LD) - gold standard
- DDC inhibitor - carbidopa (CD) enhances therapeutic benefits
- several side effects



L-Dopa

Dopamine



Main goals of the study

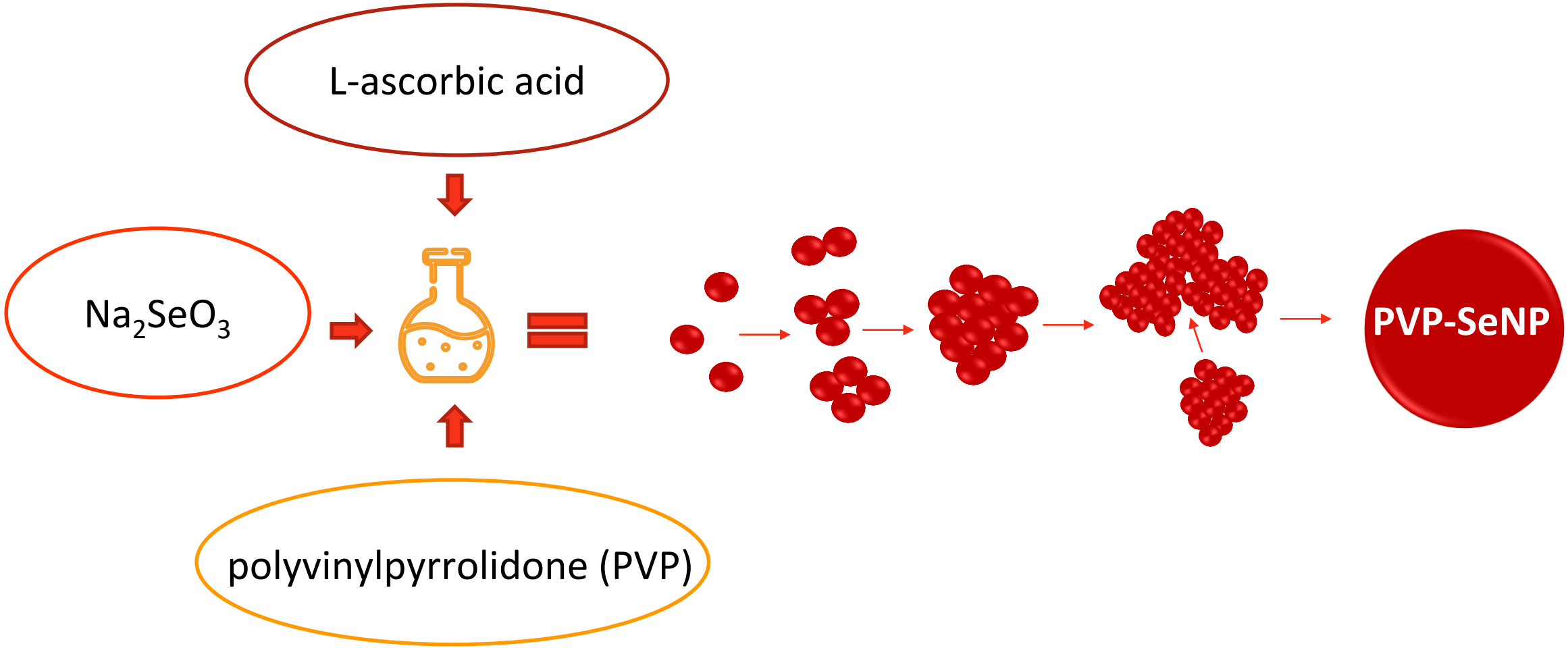
- develop multifunctional drug delivery systems for the brain
- examine their interactions with LD

- evaluate:

a) toxic effects of drug delivery system with or without LD *in vitro* and *in vivo*

b) efficacy of drug delivery system *in vitro* and *in vivo*

Selenium nanoparticles as drug carriers



1

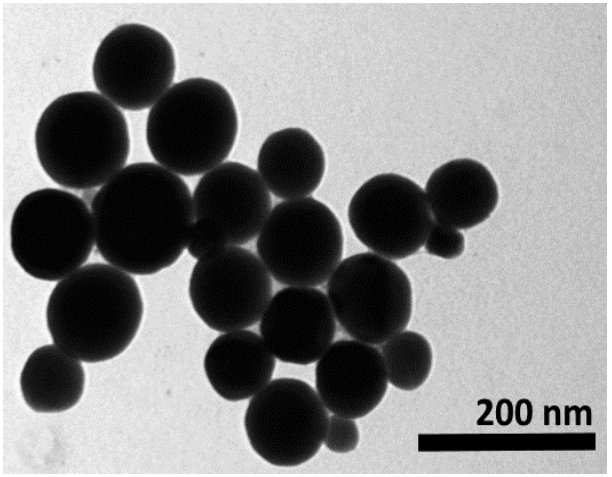
Transmission electron microscopy (TEM) – **shape and primary size (d_{TEM})**

2

Dynamic light scattering (DLS) – **hydrodynamic diameter (d_H)**

3

Electrophoretic light scattering (ELS) – **zeta (ζ) potential**



d_{TEM} / nm	d_H / nm (% intensity)	ζ potential /mV
79 ± 7	82.9 ± 0.5 (100 %)	-28.0 ± 1.9

In vivo experiment design

1. Repeated dose toxicity

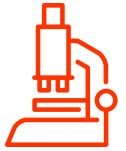
- 5 groups
- 6 males and 6 females per group
- 12 weeks old animals
- 28 days oral treatment administration

1) control group
2) LD group
3) PVP-SeNPs low dose
4) PVP-SeNPs high dose
5) PVP-SeNPs + LD

- Brain
- Kidney
- Heart
- Liver
- Blood

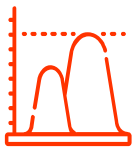
Oxidative stress evaluation

Histopathology



Blood analysis

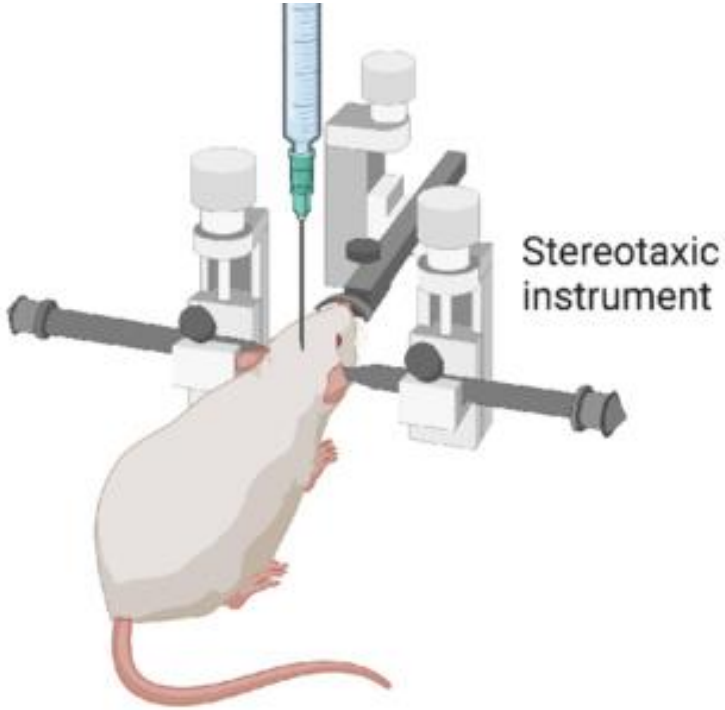
Inductively coupled plasma mass spectrometry



2. Parkinson's disease model testing

a) Stereotaxic surgery

- 6-hydroxydopamine - death of dopaminergic neurons
- generation of Hemiparkinsonian Rats
- behavioural assays



b) Drug testing

- 5 groups
- 6 males and 6 females per group
- 12 weeks old animals
- 28 days oral treatment administration – 4 weeks after surgery

1) control group
2) LD group
3) LD + CD group
4) PVP-SeNPs + LD + CD
5) PVP-SeNPs + LD

- Brain
- Kidney
- Heart
- Liver
- Blood

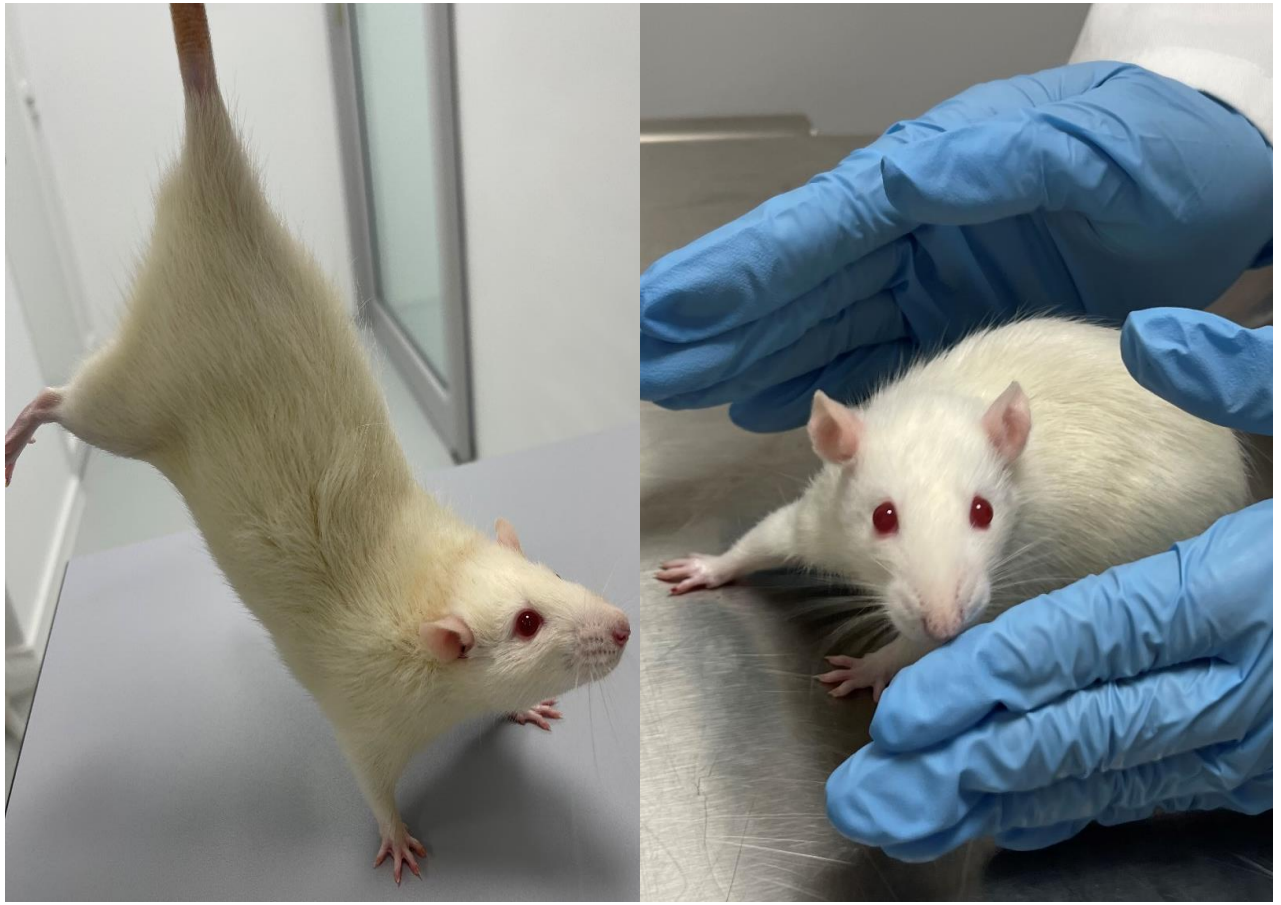
Behavioural assays

1. Rotarod performance test

- motor coordination assessing
- 2 days conditioning before experiment
- accelerating from 4 to 40 rpm
- 5 times in session with 20 minutes breaks
- time on the rod

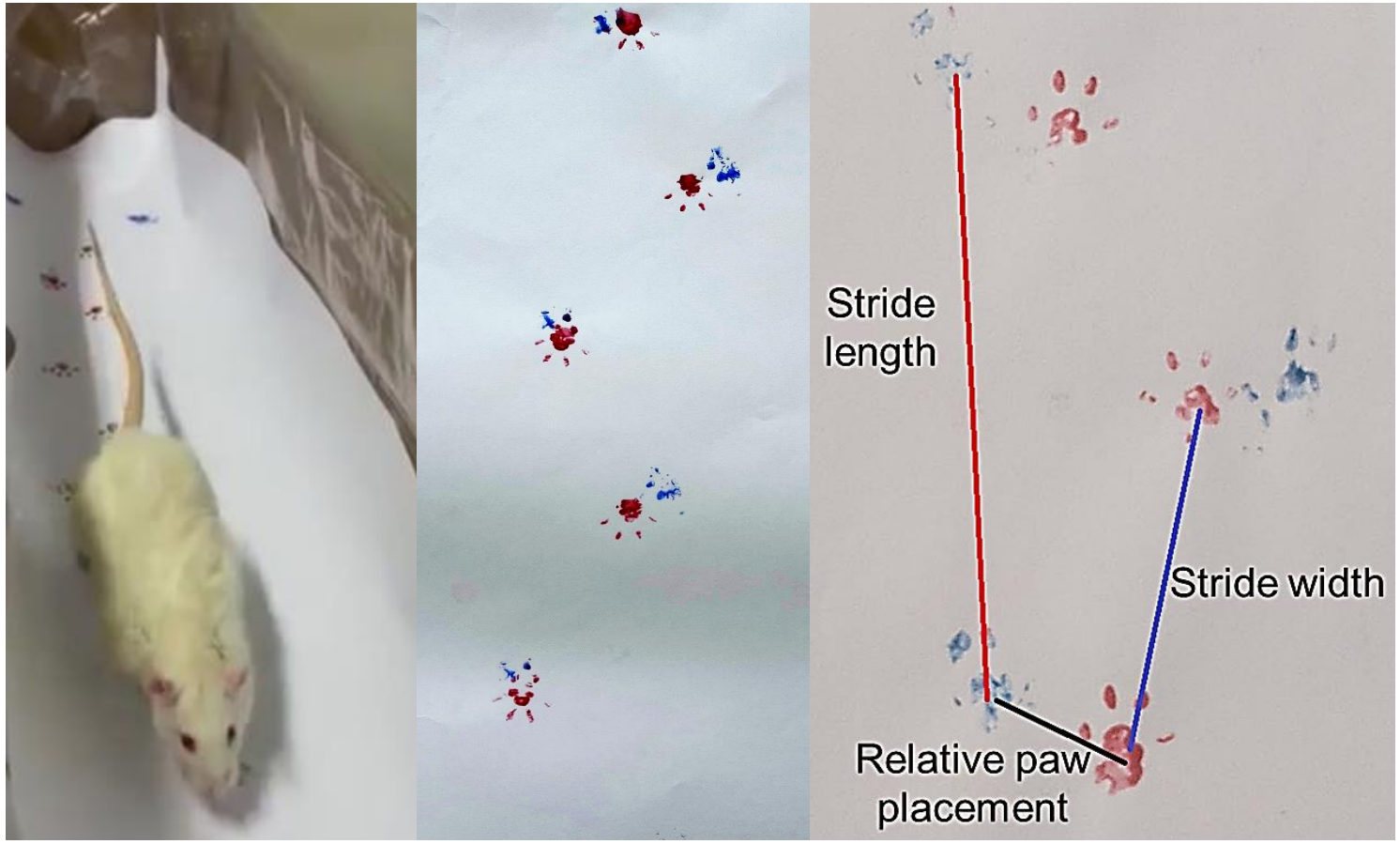
2. Neurological severity score

- hindlimb extension reflexes
- front limb extension reflexes
- resistance for lateral pulsion for the right and left side
- scores from 0 (maximal deficits) to 4 (normal) - combining scores of all test (0 to 16)



3. Gait analysis

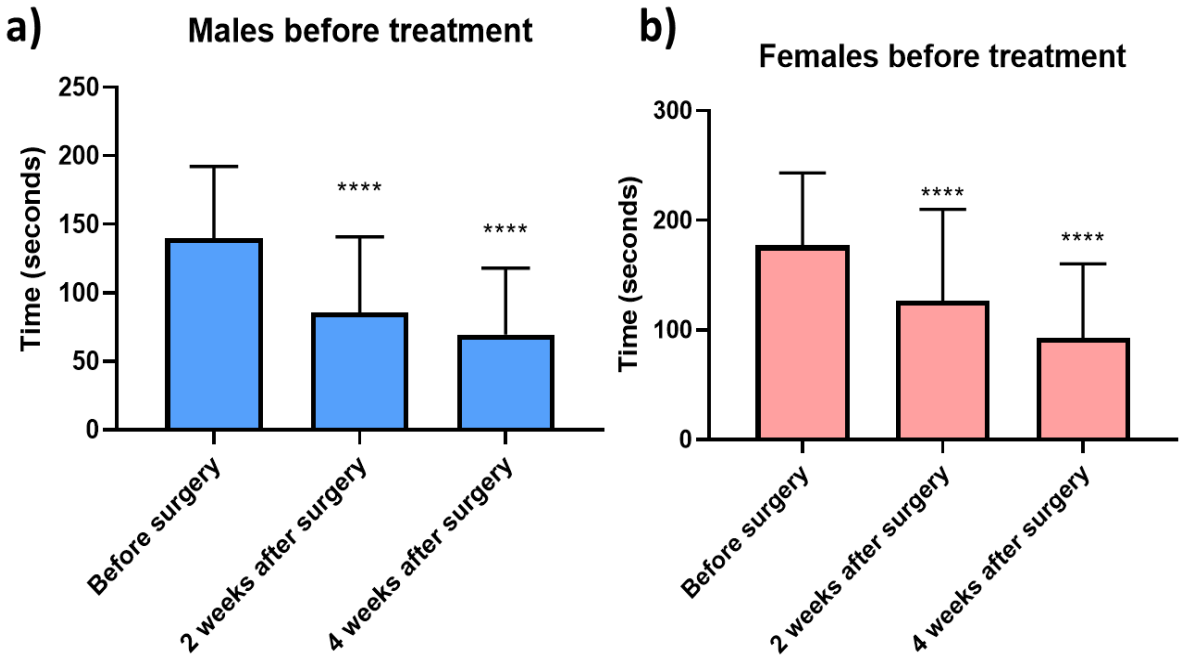
- changes in motor impairments
- 120 cm long and 20 cm wide walkway with dark goal box
- stride length and width
- relative paw placement

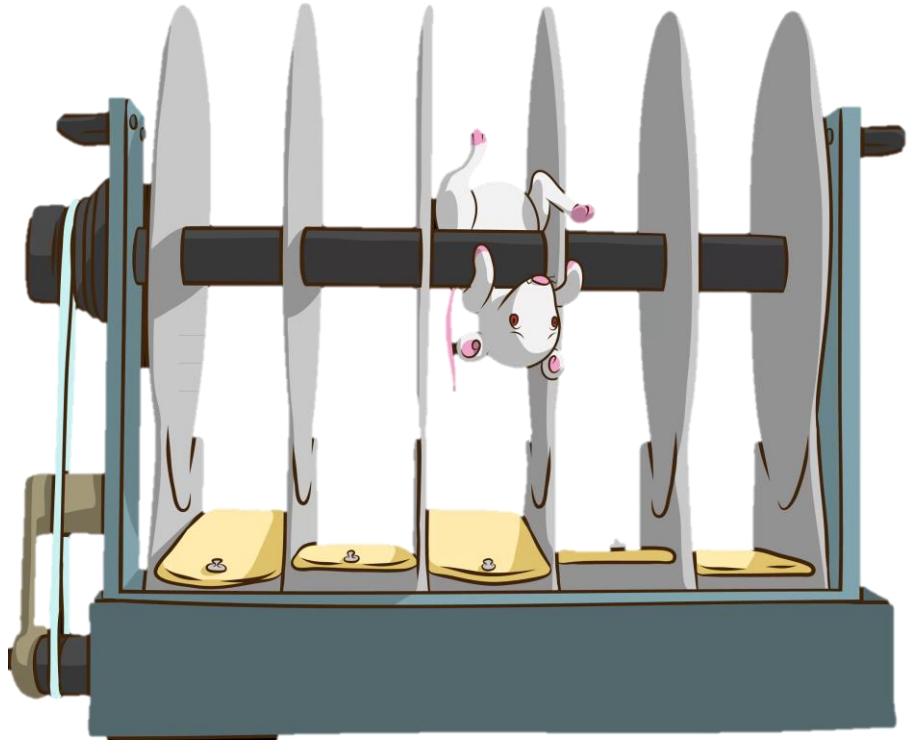


Results

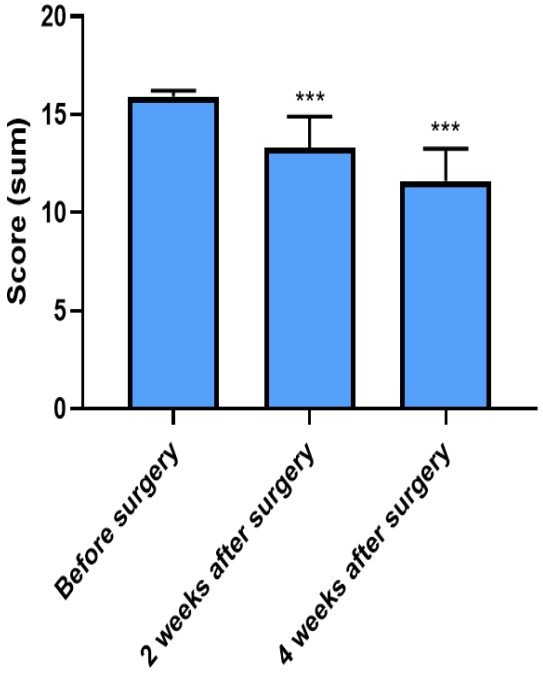
1. Behavioural tests before treatment

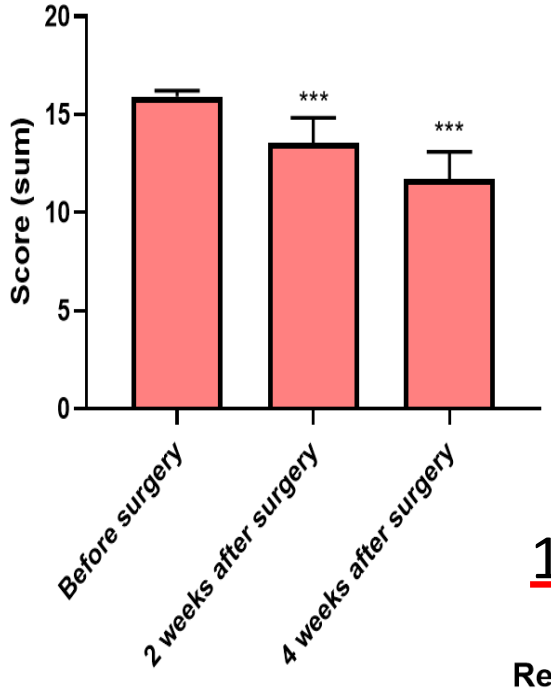
1.1. Rotarod performance



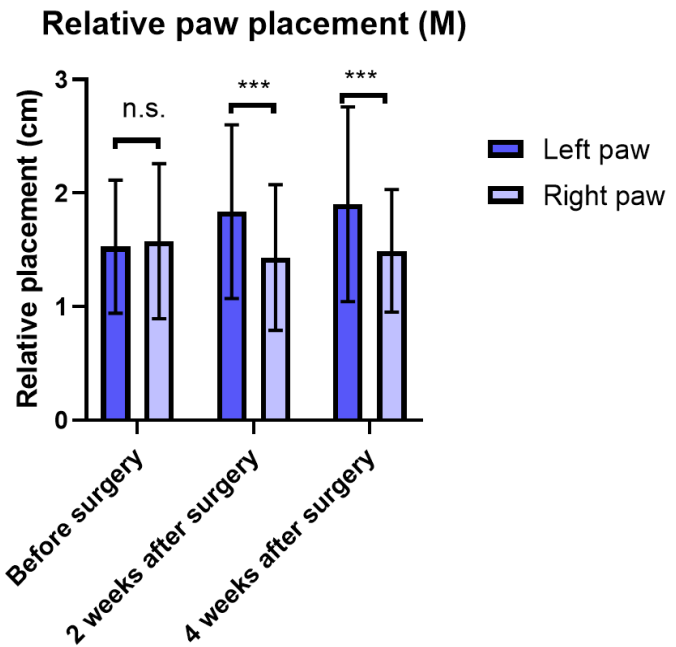


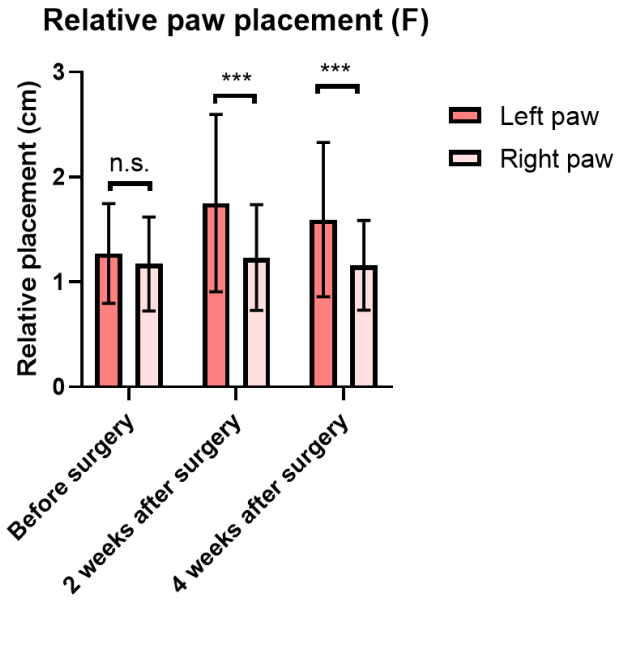
1.2. Neurological severity score





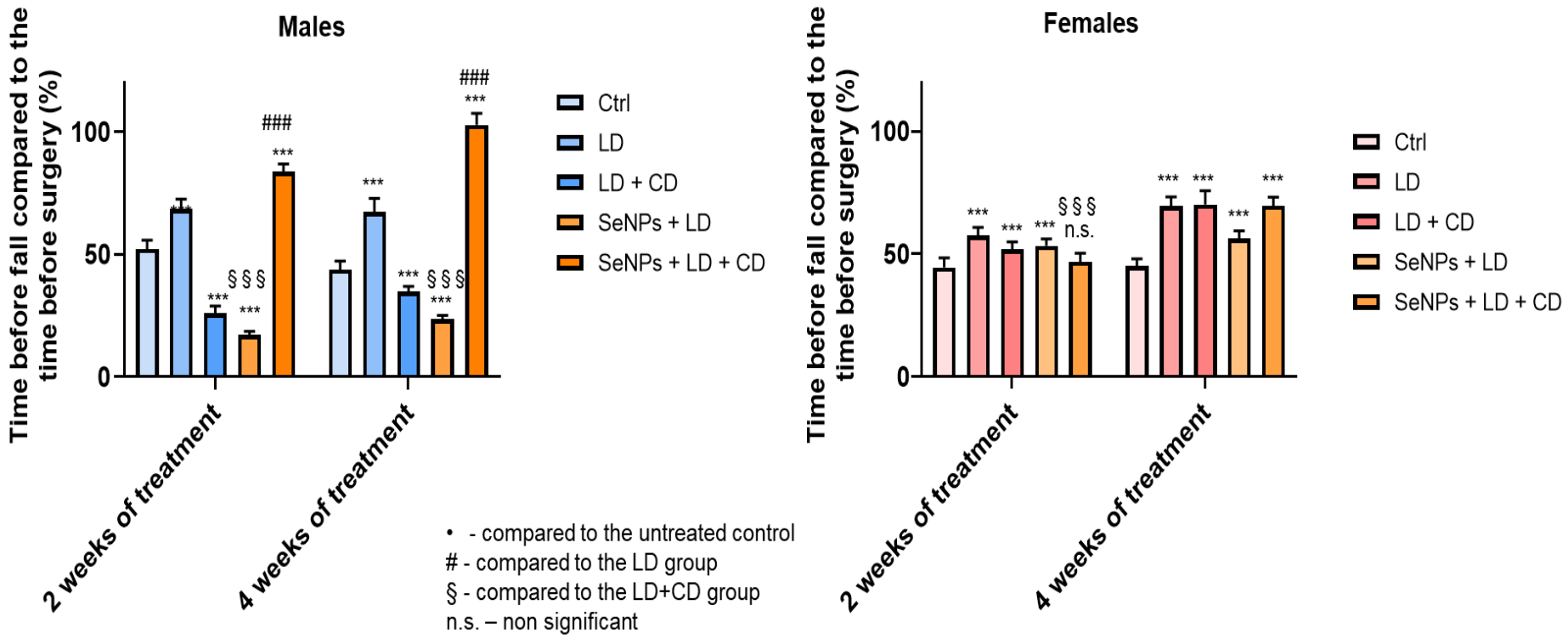
1.3. Gait analysis



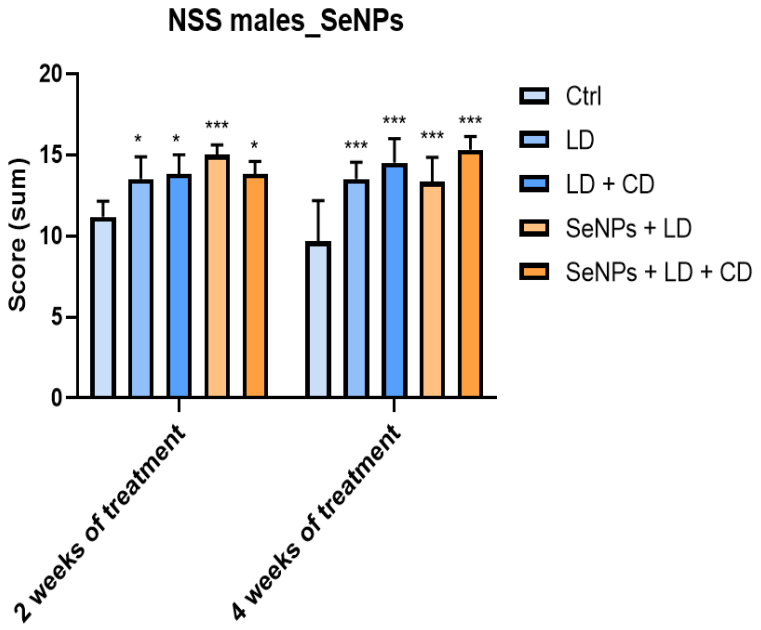


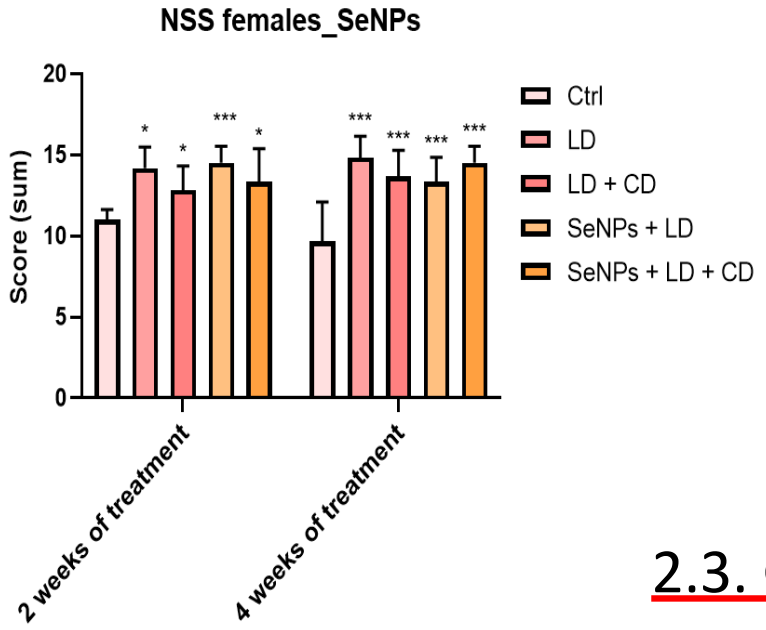
2. Behavioural tests after treatment

2.1. Rotarod performance

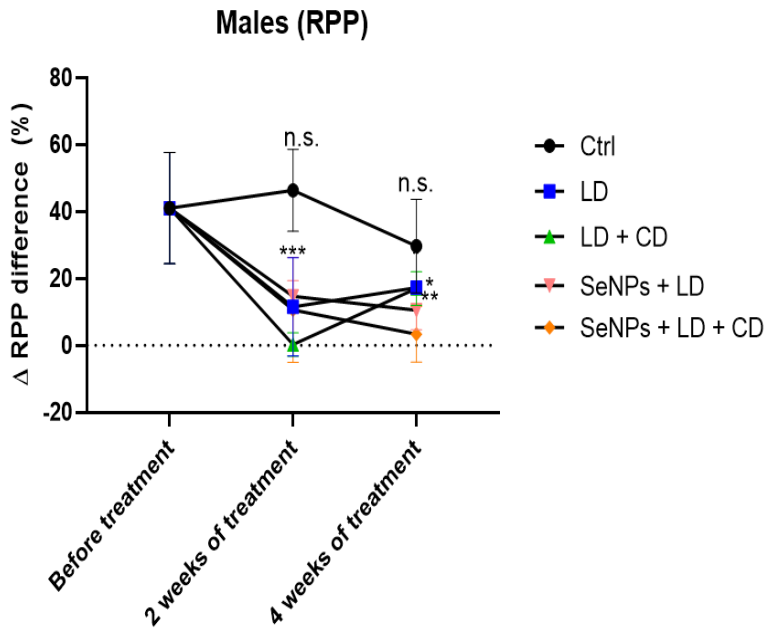


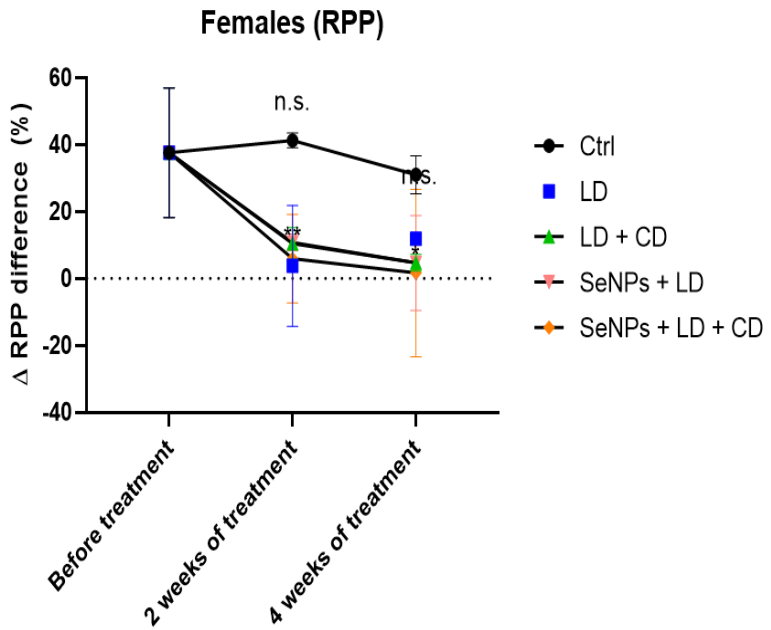
2.2. Neurological severity score



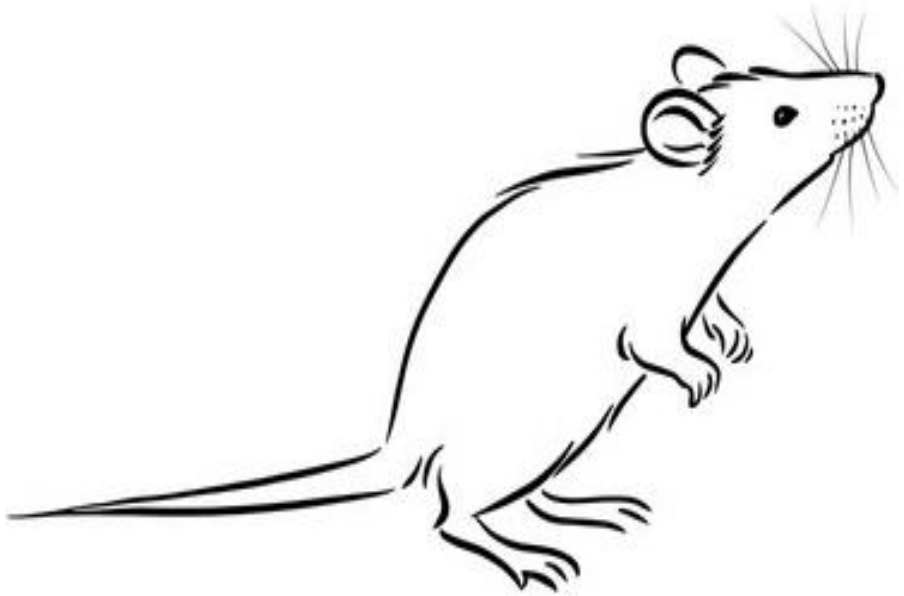


2.3. Gait analysis





Conclusions



- behavioural assays confirmed PD symptoms in rats 4 weeks after the surgery
- LD and LD + CD treated groups displayed improvements of measured conditions
- treatment with PVP-SeNPs in the combination with LD (+ CD) had a greater effect in males

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All animal experiments were **approved** by the Institutional Animal Care and Use Committee and were **in accordance with the ethical codex** for the animal welfare of the Croatian Society for Laboratory Animal Science and with international standards.