STUDY OF BLARCAMESINE IN A NEW PARKINSON'S MODEL OF COMBINED ALPHA-SYNUCLEIN PATHOLOGY AND NORADRENERGIC DYSFUNCTION

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Background

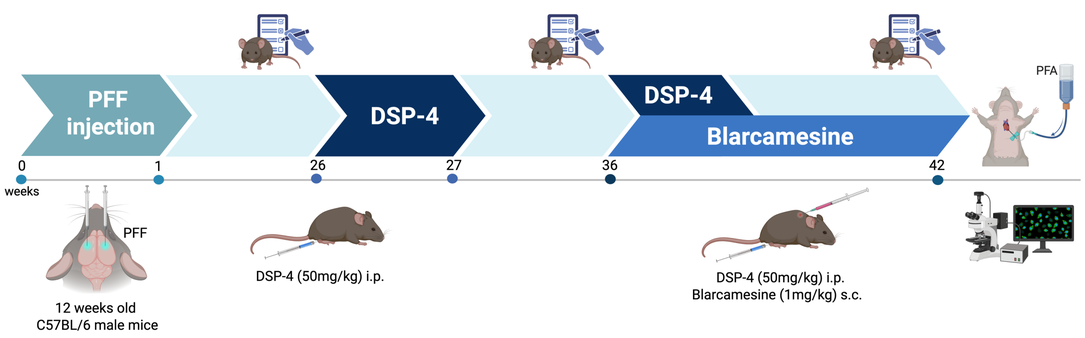
Parkinson's disease (PD) is marked by the profound degeneration of dopaminergic neurons in the substantia nigra, accompanied by the accumulation of intraneuronal protein inclusions known as Lewy bodies and Lewy neurites [1]. These inclusions are rich in fibrillar alpha-synuclein, which is believed to disrupt cellular homeostasis and elicit an inflammatory response in surrounding glial cells [2].

It seems well-known that the disruption of the noradrenergic system is linked to the onset and progression of neurodegenerative diseases. It has been demonstrated that the locus coeruleus undergoes an approximately 60% neuronal loss in patients suffering from Parkinson's disease and Alzheimer's disease even from the early stage of the disease [3].

Aim of the study

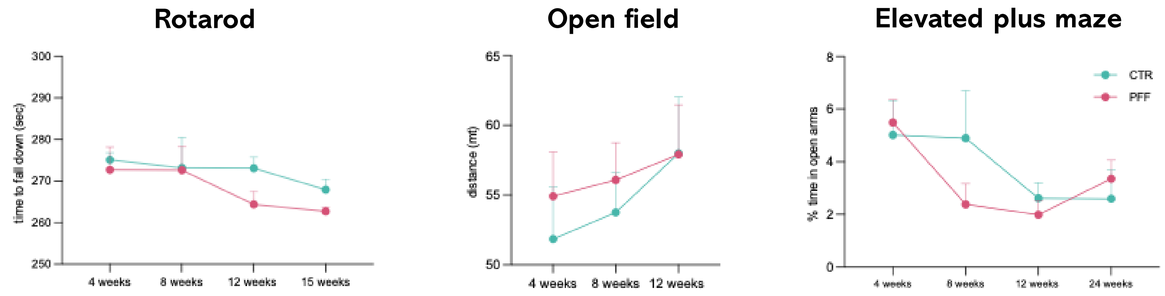
To evaluate the therapeutic potential of the sigma-1 receptor agonist blarcamesine in a new mouse model of Parkinson's disease featuring motor deficits linked to α-synuclein-driven pathology combined with noradrenergic dysfunction.

Study design and methods

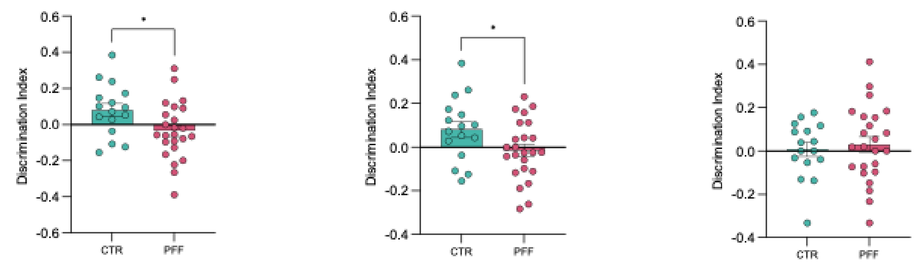


- 12-weeks old C57BL/6JRj mice injected bilaterally in the striatum with **preformed fibrils of mouse α-Syn** (PFF) (5mg/mL) provided by K. Luk [4-6].
- DSP-4 (50mg/kg) was systemically injected twice (1 week apart) 26 weeks after PFF. Mice received also a boost injection 10 weeks later.
- Mice were treated subcutaneously daily for 6 weeks with blarcamesine (1mg/kg) and tested using a battery of behavioural tests to evaluate cognitive, motor and anxiety-like behaviour.
- 42 weeks after PFF injections, mice were euthanised and perfusion-fixed with 4% ice-cold paraformaldehyde (PFA). Brains were dissected for histological analyses.

Results pre DSP-4 injection

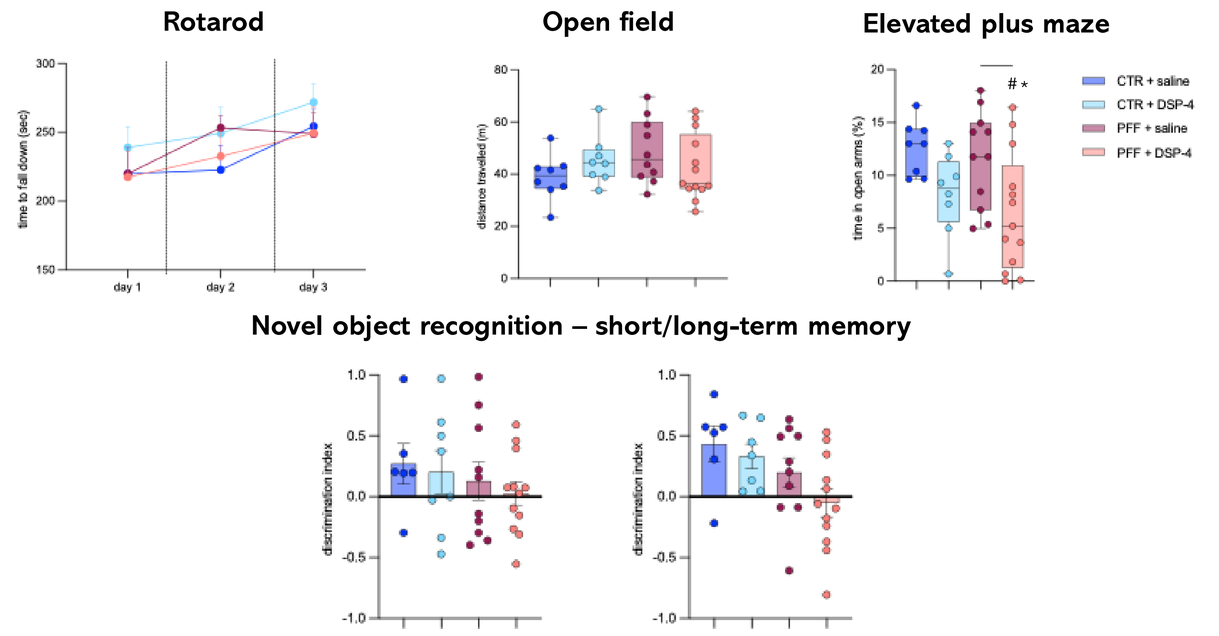


Novel object recognition – short-term memory (12, 15 and 24 weeks)



Motor coordination evaluated with rotarod test, exploratory activity and locomotion with open field test, anxiety-like behaviour with elevated plus maze test and cognitive performances with noel object recognition test.
Statistical comparisons: **(Rotarod)**. 2-way ANOVA. Time: F (1.441, 5.764) 2,208, p = 0.1938; Group: F (1, 4) 1,247, p < 0.3266; Interaction: F (1.441, 5.764) 0,4498, p < 0.5982. **(Open field)**. 2-way ANOVA. Time: F (1.911, 68,80) 2,284, p = 0.1118; Group: F (1, 38) 0,1887, p < 0.6664; Interaction: F (1.911, 68.80) 0,3486 p < 0.6972. **(EPM)**. 2-way ANOVA. Time: F (2.647, 100.6) 4,018, p = 0.0125; Group: F (1, 38) 0,3266, p < 0.5710; Interaction: F (2.647, 100.6) 0,1386 p < 0.2533. **(NOR 12 weeks)**. Unpaired t-test. p < 0.0249. **(NOR 15 weeks)**. Unpaired t-test. p < 0.0413. **(NOR 24 weeks)**. Unpaired t-test. p < 0.6563. post hoc multiple comparison (Tukey's test): * p < 0.05 versus CTR.

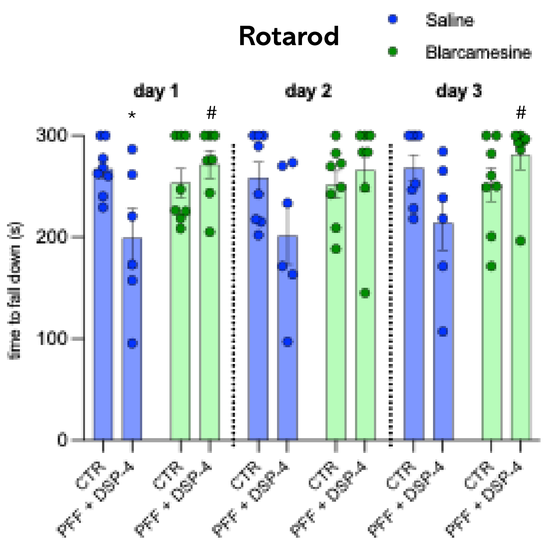
Results post DSP-4 injection

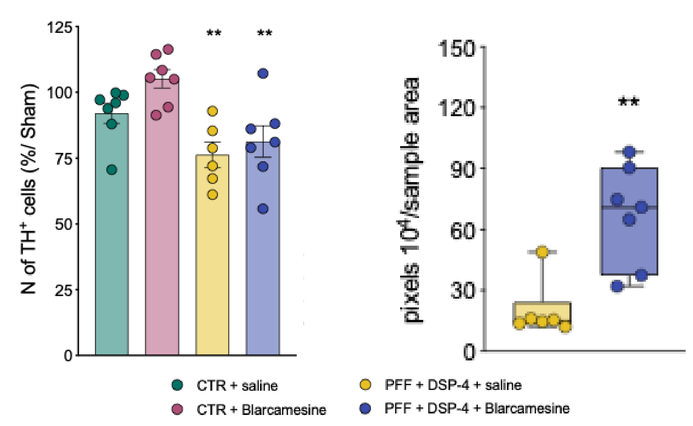


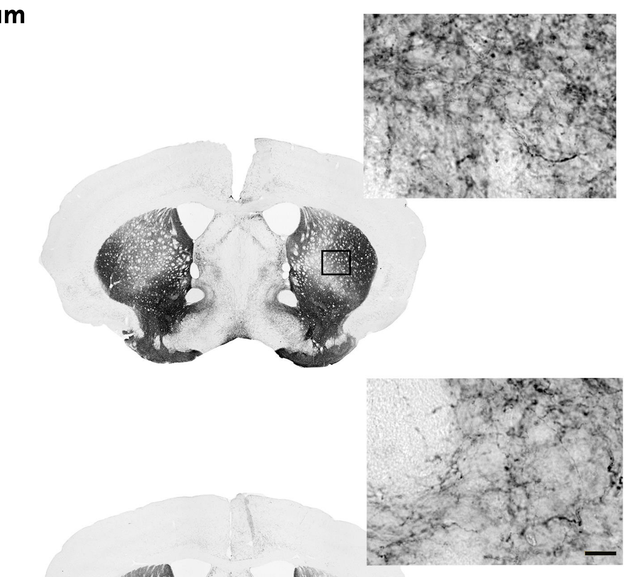
Novel object recognition – short/long-term memory

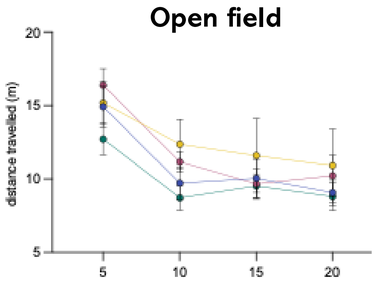
Motor coordination evaluated with rotarod test, exploratory activity and locomotion with open field test, anxiety-like behaviour with elevated plus maze test and cognitive performances with noel object recognition test.
Statistical comparisons: **(Rotarod)**. 2-way ANOVA. Time: F (2, 72) 8.558, p = 0.0005; Group: F (3, 36) 0,3861, p < 0.7636; Interaction: F (6, 72) 0,6386, p < 0.6989. **(Open field)**. One-Way ANOVA. F (1.255), p > 0,05; Tukey's multiple comparisons test: p= 0,3047. **(EPM)**. One-Way ANOVA. F (4.195), p < 0.0121; Tukey's multiple comparisons test: * p = 0,0299 CTR + saline vs. PFF + DSP-4; # p = 0,0446 PFF + DSP-4 vs. PFF + saline. **(NOR)** One-Way ANOVA. F (0.5074), p = 6800; Tukey's multiple comparisons test.

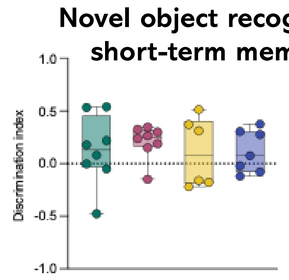
Results post blarcamesine treatment

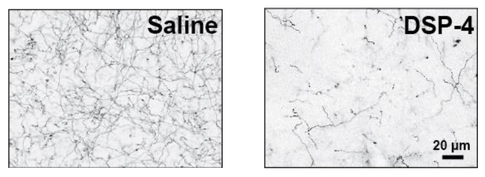












Motor coordination evaluated with rotarod test, exploratory activity and locomotion with open field test and cognitive performances with novel object recognition test. Analysis of TH-immunopositive fibers in the striatum and stereological count of dopaminergic neurons in the SNc. Representative photomicrographs of norepinephrine transporter-positive fibers in the cortex. Immunostained sections for phosphorylated alpha-synuclein.
Statistical comparisons: **(Rotarod).** 2-way ANOVA, mixed-effect analysis. Lesion: F (1, 13) 1.423; Treatment: F (1.382, 17.14) 2,136, p = 0.2765; Interaction: F (1.382, 17.14) 4.276, p = 0.2765. **Day 1.** One-Way ANOVA. F (3.006), p < 0,0492; Tukey's multiple comparisons test: * p = 0,0393 CTR + saline vs. PFF + DSP-4 + saline; # p = 0.0324 PFF + DSP-4 + Blarcamesine vs. PFF + DSP-4 + saline. **Day 2.** One-Way ANOVA. F (2.027), p < 0,1358; Holm-Sidak's multiple comparisons test. **Day 3.** One-Way ANOVA. F (3.021), p < 0,0485; Tukey's multiple comparisons test: # p = 0,0430 PFF + DSP-4 + Blarcamesine vs. PFF + DSP-4 + saline. **(Open field)**. 2-way ANOVA. Time: F (2.248, 56.19) 42.72, p < 0.0001; Group: F (3, 25) 0,9654, p = 0.4245; Interaction: F (6.743, 56.19) 1.189, p = 0.3245. **(NOR)**. One-Way ANOVA. F (0.2385), p = 0.8687; Tukey's multiple comparisons test. **(TH dopamine cells SNc)**. One-way ANOVA. F (0.4679) , p < 0.0011. Tukey's multiple comparisons test. ** p < 0.0014 CTR + Blarcamesine vs. PFF + DSP-4 + saline; ** p < 0.0061 CTR + Blarcamensine vs. PFF + DSP-4 + Blarcamesine. **(TH striatum fiber density)**. Mann-Whitney test. p < 0.0047. Scale bar TH striatum = 5 mm and 10 µm (inset). Scale bar pSyn = 5 mm and 100 µm. (inset).

References

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[3] Gargano et al., Front. Neurosci, 2023;
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[5] Kelvin Luk et al., Science 2012;338(6109):949-53
[6] Paxinos et al.,2001

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Conclusions

In the PFF-DSP-4 double hit Parkinson's mouse model, blarcamesine showed **promising effects with rapid kinetics on motor performance**, which was *completely* rescued after 6 weeks of treatment and with **protracted effect on fiber density** of tyrosine-hydroxylase+ fibers in the striatum, additionally **suggesting blarcamesine as a true neuroprotectant**. These new findings are very promising and provide scientific support to blarcamesine's emerging profile as a **candidate disease-modifying therapeutic for Parkinson's disease.**