Research Article

Neuroprotective and anti-inflammatory effects of a therapy combining agonists of nicotinic α7 and σ1 receptors in a rat model of Parkinson's disease

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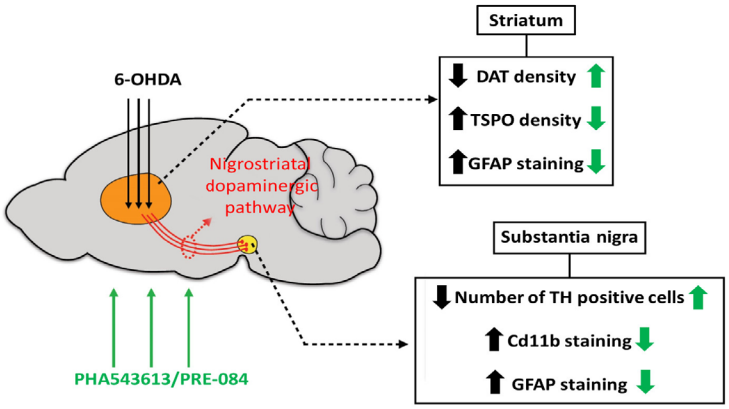
Graphical Abstract *Dual administration of PHA543613 (agonist of α7 nicotinic receptor) and PRE-084 (agonist of σ1 receptor) induces a partial protection of the nigro-striatal dopaminergic pathway and a reduction of glial cells activation in a rat model of Parkinson's disease*



Abstract

To date there is no treatment able to stop or slow down the loss of dopaminergic neurons that characterizes Parkinson's disease. It was recently observed in a rodent model of Alzheimer's disease that the interaction between the α7 subtype of nicotinic acetylcholine receptor (α7-nAChR) and sigma-1 receptor (σ1-R) could exert neuroprotective effects through the modulation of neuroinflammation which is one of the key components of the pathophysiology of Parkinson's disease. In this context, the aim of the present study was to assess the effects of the concomitant administration of N-(3R)-1-azabicyclo[2.2.2]oct-3-yl-furo[2,3-c]pyridine-5-carboxamide (PHA) 543613 as an α7-nAChR agonist and 2-(4-morpholinethyl) 1-phenylcyclohexanecarboxylate (PRE)-084 as a σ1-R agonist in a well-characterized 6-hydroxydopamine rat model of Parkinson's disease. The animals received either vehicle separately or the dual therapy PHA/PRE once a day until day 14 post-lesion. Although no effect was noticed in the amphetamine-induced rotation test, our data has shown that the PHA/PRE treatment induced partial protection of the dopaminergic neurons (15–20%), assessed by the dopamine transporter density in the striatum and immunoreactive tyrosine hydroxylase in the substantia nigra. Furthermore, this dual therapy reduced the degree of glial activation consecutive to the 6-hydroxydopamine lesion, i.e, the 18 kDa translocation protein density and glial fibrillary acidic protein staining in the striatum, and the CD11b and glial fibrillary acidic protein staining in the substantia nigra. Hence, this study reports for the first time that concomitant activation of α7-nAChR and σ1-R can provide a partial recovery of the nigro-striatal dopaminergic neurons through the modulation of microglial activation. The study was approved by the Regional Ethics Committee (CEEA Val de Loire n°19) validated this protocol (Authorization N°00434.02) on May 15, 2014.

Key Words: 6-hydroxydopamine; astrocytes; microglial activation; neurodegeneration; neuroinflammation; nicotinic α7 receptor; Parkinson's disease; PHA 543613; PRE-084; sigma-1 receptor

Chinese Library Classification No. R453; R364; R741

Introduction

Parkinson's disease (PD) is the second most frequent age-related neurodegenerative disorder after Alzheimer's disease (AD), and to date treatments able to stop or slow down its progress are yet to be found. The main feature of PD is the progressive death of dopaminergic (DA) neurons located in the substantia nigra pars compacta (SNpc). This neurodegeneration induces gradual dopamine depletion in the striatum giving rise to motor symptoms including resting tremor, rigidity, akinesia and postural instability, which appear

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when approximately 50–60% of nigrostriatal DA neurons have been lost. Besides these motor signs, a wide range of non-motor symptoms such as dysfunction of the gastrointestinal and autonomic nervous system, and cognitive impairments have been identified (Kalia and Lang, 2015; Schapira et al., 2017). Neuroinflammation is also an important feature of PD, resulting in the activation of microglia and astrocytes in the SNpc, thus inducing the release of pro-inflammatory cytokines and reactive oxygen species that increase DA neuron death (Tansey et al., 2007).

As recently reviewed, epidemiologic studies have shown that the prevalence of PD seems to be lower in smokers than in non-smokers, and have suggested that nicotine may underlie this beneficial effect (Chen, 2018). In parallel, it was shown that nicotine exhibited neuroprotective effects in rodent models of PD (Janson et al., 1988; Costa et al., 2001). These effects were also obtained using various α7 nicotinic acetylcholine receptor (α7-nAChR) agonists, thus demonstrating its major role in this neuroprotection (Suzuki et al., 2013; Bordia et al., 2015). Other preclinical experiments have suggested that the neuroprotective effects exerted by α7-nAChR activation could be related to the modulation of neuroinflammation associated with the degeneration of DA neurons. This was assessed in a mouse model of PD induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration (Liu et al., 2012; Stuckenholz et al., 2013), and in a 6-hydroxydopamine (6-OHDA)-induced rat model of PD (Sérrière et al., 2015), and it is noteworthy that in all these experiments the preservation of DA neurons was only partial.

Besides α7-nAChR, other pharmacological targets have emerged as potentially beneficial in the management of PD such as the sigma-1 receptor (σ1-R). This receptor is located in many peripheral organs as well as in the brain, and it is involved in virtually all central nervous system disorders (Maurice and Su, 2009; Kourrich et al., 2012). The activation of σ1-R was shown to have neuroprotective effects on DA function after 6-OHDA-induced lesion in rodents (Francardo et al., 2014, 2017, 2019). Several mechanisms underlying these effects such as improvement of cell survival and neuroplasticity may be involved (Ruscher and Wieloch, 2015).

In neurodegenerative conditions other than PD, various data have indicated that the interaction between α7-nAChR and σ1-R could reinforce the neuroprotective mechanisms. This was first shown with the administration of pregnenolone which, among various effects, can activate both α7-nAChR and σ1-R, and has had a beneficial impact on cognition in a mouse model of AD induced by intracerebral beta-amyloid 25–35 peptide injection (Yang et al., 2012). Subsequently, it was reported in the same animal model that the σ1-R agonist PRE-084 in combination with the acetylcholinesterase inhibitor donepezil was able to exert neuroprotective effects as assessed by various behavioral tests (Maurice, 2016). In addition, it was shown in this study that these beneficial effects were related to a synergistic mechanism involving α7-nAChR specifically, as they were prevented by the antagonist methyllycacotinine.

In this context, we hypothesized that the combination of α7-nAChR and σ1-R agonists could be a relevant lead to follow up in the search for a potent PD treatment. Therefore, we evaluated in this study the effects of the concomitant administration of PHA543163 as an α7-nAChR agonist and PRE-084 as a σ1-R agonist in a 6-OHDA-induced rat model of PD.

Materials and Methods

Animals

All experimental procedures on animals were carried out in accordance with the European Community Council Directive 2010/63/EU for Laboratory Animal Care. The Regional Ethics Committee (CEEA Val de Loire n°19) validated this protocol (Authorization N°00434.02) on May 15, 2014, and the numbers of animals used and their suffering were kept to a minimum. For this experiment, 14 male Wistar rats aged 8–9 weeks (body weight 275–300 g, Charles River, France) were used. As indicated in the ethics guidelines, the rats were accommodated in social groups of two per cage, in an environment with controlled temperature and humidity (20 ± 2°C and 55 ± 5%, respectively), a 12-hour light/dark cycle, and food and water provided *ad libitum*.

6-OHDA lesion

The surgical procedure was performed according to Vetel et al. (2019). In brief, the rats were put in a stereotaxic apparatus (Stoelting, Phymep, Paris, France) with a tooth bar set to –3.3 mm under isoflurane (Baxter, Guyancourt, France) anesthesia (4% and then 2.5% during surgery). They received a total dose of 12 µg of 6-OHDA hydrochloride (Sigma-Aldrich, Saint-Quentin Fallavier, France) in three injections each at the dose of 4 µg in 2 µL (solution of 2 mg/mL in 0.01% ascorbic acid/saline, pH 4.5) dispensed into the right striatum at the following coordinates from Bregma: Antero-posterior (AP)1 = +1.6 mm, lateral (L)1 = –2.8 mm, profoundness (P)1 = –6 mm; AP2 = 0.0 mm, L2 = –4.1 mm, P2 = –5.5 mm; AP3 = –1.2 mm, L3 = –4.5 mm, P3 = –5.5 mm (Paxinos and Watson, 2009). After surgery, a sub-cutaneous injection of buprenorphine (0.05 mg/kg; Centravet, Plancoët, France) was given to prevent postoperative pain.

Drug treatments

Treatments were carried out with the α7-nAChR agonist N-(3R)-1-azabicyclo[2.2.2]oct-3-yl-furo[2,3-c] pyridine-5-carboxamide (PHA) 543613 (ICOA, Orléans, France) and the σ-1R agonist 2-(4-morpholinethyl) 1-phenylcyclohexanecarboxylate (PRE)-084 (Tocris Biosciences, Lille, France), both dissolved in sterile water. The administration of drugs started at 4 hours post intra-striatal 6-OHDA injection and was continued daily for 14 days. PHA 543613 was given at a dose of 6 mg/kg per day per os and PRE-084 at 1 mg/kg per day through intra-peritoneal (i.p.) injection. In this study, fourteen 6-OHDA lesioned rats were randomly divided into two experimental groups of seven. The first groups received the dual therapy (PHA/PRE group) and at the same time point the other received vehicle (Control group: 0.3 mL sterile water/300 g body weight per os and 0.3 mL sterile water/300 g body weight intraperitoneally).

Amphetamine-induced rotations test

This classical test uses the presynaptic dopamine releaser amphetamine to induce ipsilateral rotations in animals with unilateral dopaminergic pathway lesion (Ungerstedt and Arbuthnott, 1970). D-amphetamine sulfate (3 mg/kg; Sigma-Aldrich, Saint-Quentin Fallavier, France) was injected intraperitoneally in all rats at 13 days post-lesion. They were then positioned in an automatic rotameter bowls (Imetronic, Pessac, France). The measurement of the number of rotations in the ipsilateral direction started 15 minutes after the drug administration and was performed for 2 hours. A complete and uninterrupted 360° turn was considered as a full rotation.

Evaluation of the effects of the PHA 543613/PRE-084 dual therapy by autoradiography and immunofluorescence analysis

Brain tissue preparation

The rats were euthanatized at 14 days post-lesion by the transcardiac perfusion of 250 mL of cold heparinized physiological saline under deep anesthesia with sodium pentobarbital (i.p., 60 mg/kg, Ceva Santé Animale, Libourne, France). The brains were then removed, tap frozen at –35°C using isopentane, and stored at –80°C. Sixteen-µm coronal

brain sections were then cut at –20°C (cryostat CM30550S, Leica, Biosystems, Nanterre, France), collected on gelatinized slides, and then stored at –80°C until the experiments.

Quantification of dopamine transporter and translocator protein by autoradiography

The binding of [^{125}I]PE2I to the dopamine transporter (DAT) which quantifies the density of dopaminergic nerve terminals, and the binding of [^{3}H]PK-11195 to the 18 kDa translocator protein (TSPO) which is overexpressed by activated microglia, were measured in the striatum. [^{125}I]PE2I (molar activity 95 GBq/µmol, obtained according to Vetel et al., 2019) and [^{3}H]PK-11195 (molar activity 3.06 GBq/µmol, Perkin Elmer, Norwalk, CT, USA) were used according to Maia et al. (2012). Six adjacent brain sections per rat were used with each radioligand. Non-specific binding was measured using 100 µM of cocaine chlorhydrate (Cooper Industrie, Melun, France) or 1 µM of stable PK-11195 (Sigma-Aldrich, Saint-Quentin, Fallavier, France) for [^{125}I]PE2I and [^{3}H]PK-11195 experiments, respectively. The β-imager™ 2000 system (Biospace Lab, Paris, France) equipped with the β-vision software (M3-Vision™ Biospace Instruments, Paris, France) was used for autoradiogram quantification in the contralateral (CL) non-lesioned striatum and ipsilateral (IL) lesioned striatum (C-ST and I-ST, respectively), according to Vetel et al (2019). The specific binding (total minus non-specific binding) was expressed as counts per minute per square millimeter (cpm/mm^2).

Evaluation of DA neuron survival (TH), microglial (CD11b) and astroglial (GFAP) activation by immunofluorescence

Immunostaining experiments were performed for tyrosine hydroxylase (TH) in the SNpc as a marker of DA neurons, CD11b in the SNpc as a marker of microglia, and glial fibrillary acidic protein (GFAP) both in the striatum and SNpc as a marker of astrocytes. For each immunostaining and each rat, two consecutive coronal brain sections were used.

After fixation in 4% paraformaldehyde solution (Sigma-Aldrich), blocking and permeabilization of sections were performed before overnight incubation of primary antibodies at 4°C (**Table 1**). Sections were then incubated with fluorescence-coupled secondary antibodies (1 hour, room temperature; **Table 1**) and the nuclei were counterstained with 4′ 6-diamidino-2-phenylindole (DAPI, 1:10,000; Sigma-Aldrich). The fluorescence signal was collected in at least two areas for each section with a fluorescence microscope (Leica DM5500 B) connected to a CCD camera (ORCA-R2, Hamamatsu Photonics, Massy, France). The ImageJ software (https://imagej.nih.gov/ij/) was used to analyze images.

For dopaminergic neurons (TH staining), the number of TH$^+$ cells was manually counted from pictures both in the ipsilateral and contralateral substantia nigra (I-SN and C-SN, respectively). For CD11b and GFAP in the SN, the percentage of area stained was automatically assessed in regions of interest delimitating the ipsi- and contralateral sides. For GFAP in the I-ST and C-ST, the percentage of stained area was automatically measured in the picture. The areas analyzed for each structure are represented in **Figure 1**.

Data analysis

The results were expressed as the mean ± standard deviation (SD). The statistical analysis between the I-ST and C-ST or between the I-SN and C-SN was performed with the Wilcoxon test. In autoradiography and immunohistochemistry experiments, the relative difference (%) in values between the ipsilateral (IL, lesioned) and contralateral (CL, non-lesioned) hemispheres was calculated as: (IL–CL)/CL × 100. The Mann-Whitney *U* test was applied to compare PHA/PRE group *versus* control group. All were analyzed with the GraphPad Prism 6 software (GraphPad Software Inc., La Jolla, CA, USA) and the level of significance was set at $P < 0.05$.

Table 1 | **Antibodies used for immunohistochemistry analysis**

Target	Primary antibody	Secondary antibody
TH	Rabbit polyclonal, Cat# ab112 (Abcam, Paris, France), 1:1000	Goat anti-rabbit Alexa 555, Cat# ab150086 (Abcam), 1:500
GFAP	Rabbit polyclonal, Cat# Z0334 (DAKO, Les Ulis, France), 1:1000	
CD11b	Mouse monoclonal clone, Cat# CBL1512 (Merck Millipore, Fontenay-sous-Bois, France), 1:500	Goat anti-mouse Alexa 488, Cat# ab150113 (Abcam), 1:500

GFAP: Glial fibrillary acidic protein; TH: tyrosine hydroxylase.

Results

Amphetamine-induced rotations

No significant difference between the number of ipsilateral rotations in the control group ($n = 7$) and PHA/PRE group ($n = 7$) was observed at 13 days post-lesion (8.68 ± 3.80 *vs.* 10.06 ± 2.36 ipsilateral rotations/min, respectively, $P > 0.05$).

Dopaminergic neurons survival

DAT expression in the striatum

In each experimental group (Control and PHA/PRE), the DAT density was measured by quantitative autoradiography in the C-ST and I-ST (**Figure 2A**). In the C-ST, the [^{125}I]PE2I binding was homogenous between the control and PHA/PRE group ($P > 0.05$). In the I-ST, this binding was significantly decreased *versus* C-ST in the control group ($P < 0.05$, Wilcoxon test) and PHA/PRE group ($P < 0.05$, Wilcoxon test) (**Figure 2B**). The relative decrease in DAT density in the lesioned (I-ST) *versus* non-lesioned (C-ST) striatum was then 78% in the control group and 64% in the PHA/PRE group. The difference between I-ST in the PHA/PRE *versus* the control group was statistically significant ($P < 0.05$, Mann-Whitney *U* test).

TH expression in the substantia nigra

The immunoreactive TH was evaluated in both experimental groups as an index of DA cell bodies (**Figure 2C**). As represented in **Figure 2D**, quantitative measurements in the C-SN showed that the number of TH positive cells was homogenous between the control and PHA/PRE group ($P > 0.05$). In the I-SN, this number was significantly decreased in both the control ($P < 0.05$, Wilcoxon test) and PHA/PRE group ($P < 0.05$, Wilcoxon test). The relative decrease in TH positive cells in the lesioned (I-SN) *versus* intact (C-SN) SN was then 73% in the control group and 53% in the PHA/PRE group. The number of TH positive cells in the I-SN was significantly higher in the PHA/PRE group than in the control group ($P < 0.001$, Mann-Whitney *U* test).

Neuroinflammation

Glial activation in the striatum

For microglia activation, the TSPO density was assessed by autoradiography using [^{3}H]PK-11195 binding in the C-ST and I-ST in the control and PHA/PRE groups (**Figure 3A**). The quantitative measurements in the C-ST showed that the [^{3}H]PK-11195 binding was homogenous between the two experimental groups ($P > 0.05$; **Figure 3B**). In the I-ST, this binding was significantly increased *versus* C-ST in the control ($P < 0.05$, Wilcoxon test) and PHA/PRE groups ($P < 0.05$, Wilcoxon test). The relative increase in TSPO density in the lesioned *versus* intact striatum was then 73% in the control group and only 53% in the PHA/PRE. However, the difference between I-ST in the PHA/PRE compared to the control group was not statistically significant ($P > 0.05$).

Astrocytes activation was assessed by GFAP immunohistochemistry (**Figure 3C**). The GFAP staining (% area occupied) was low and similar among the groups in the C-ST ($P > 0.05$; **Figure 3D**). In

the I-ST, area occupied by GFAP staining in the striatum was significantly increased *versus* C-ST in both the control ($P < 0.05$, Wilcoxon test) and PHA/PRE group ($P < 0.05$, Wilcoxon test). The relative increase in GFAP staining in the lesioned *versus* intact ST was then 158% in the control group and 135% in the PHA/PRE group. The difference between I-ST in the PHA/PRE compared to the control group was statistically significant ($P < 0.05$, Mann-Whitney *U* test).

Glial activation in the substantia nigra

Microglial activation in the SN was investigated through CD11b expression in both experimental groups (**Figure 4A**). Quantitative measurements in the C-SN (% area occupied) in both groups were very low and under the detection threshold (**Figure 4B**). In the I-SN, this staining was detectable in the control and PHA/PRE groups, with a significant statistical difference between both ($P < 0.001$, Mann-Whitney *U* test).

Astrocyte activation was assessed by GFAP immunohistochemistry (**Figure 4C**). The GFAP staining (% area occupied) was low and similar between the control and PHA/PRE groups in the C-SN ($P > 0.05$) (**Figure 4D**). In the I-SN, the area occupied by GFAP staining was significantly increased in both the control group ($P < 0.05$, Wilcoxon test) and PHA/PRE group ($P < 0.05$, Wilcoxon test). The relative increase in GFAP staining in the lesioned *versus* intact SN was then 140% in the control group and 132% in the PHA/PRE. The difference between I-SN in the PHA/PRE compared to the control group was statistically significant ($P < 0.05$, Mann-Whitney *U* test).

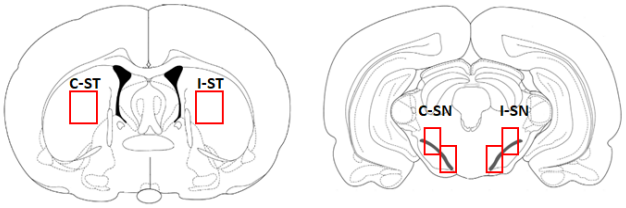

Figure 1 | **Coronal rat brain section representation.**
The red squares represent areas where pictures have been taken for immunohistochemistry analysis in the contralateral and ipsilateral striatum (C-ST and I-ST, left) and substantia nigra (C-SN and I-SN, right). Adapted from Paxinos and Watson (2009).

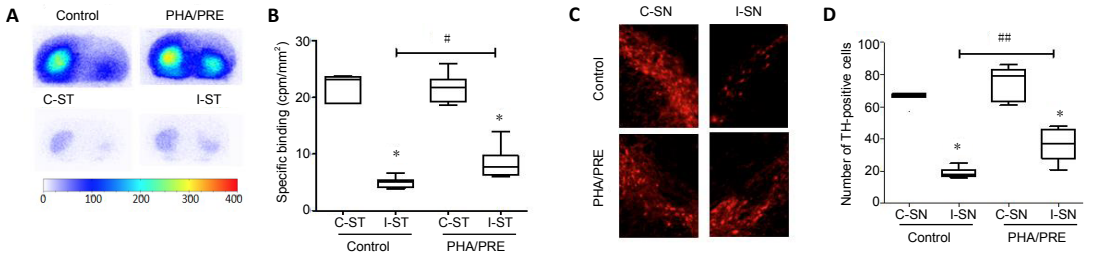

Figure 2 | **Analysis of dopaminergic neurons: DAT expression in the striatum and TH immunoreactivity in the substantia nigra.**
(A) Representative total (upper) and non-specific (lower) binding of [^{125}I]PE2I to the DAT in the non-lesioned intact (C-ST) and lesioned (I-ST) striatum of the control and PHA/PRE groups. (B) Quantification of the specific binding of [^{125}I]PE2I in the C-ST and I-ST in both groups. (C) Representative immunostaining of TH (red) in the C-SN and I-SN of the control and PHA/PRE groups. (D) Measurement of TH positive cells in the C-SN and I-SN of both groups. Data are represented as the mean ± SD. $n = 7$/group. *$P < 0.05$, *vs*. contralateral area in the same group (Wilcoxon test); #$P < 0.05$ and ##$P < 0.01$ (Mann-Whitney *U* test). C-SN: Contralateral substantia nigra; C-ST: contralateral striatum; DAT: dopamine transporter; I-SN: ipsilateral substantia nigra; I-ST: Ipsilateral striatum; TH: tyrosine hydroxylase.

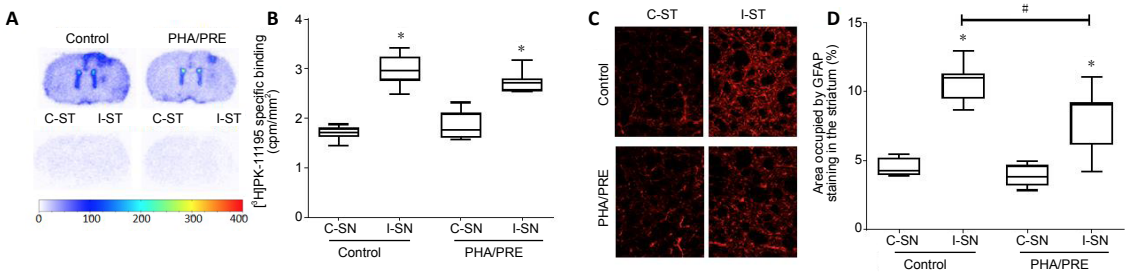

Figure 3 | **Neuroinflammation in the striatum: TSPO expression and GFAP immunoreactivity.**
(A) Representative total (upper) and nonspecific (lower) binding of [^{3}H]PK-11195 to TSPO in C-ST and I-ST in the control and PHA/PRE groups. (B) Quantification of the specific binding of [^{3}H]PK-11195 in the C-ST and I-ST in both groups. (C) Representative immunostaining of GFAP (red) in C-ST and I-ST in both groups. (D) Quantification of the expression of GFAP (% area occupied) in C-ST and I-ST in the control and PHA/PRE groups. Data are represented as mean ± SD. $n = 7$/group. *$P < 0.05$, *vs*. contralateral area in the same group (Wilcoxon test); #$P < 0.05$ (Mann-Whitney *U* test). C-ST: Contralateral striatum; GFAP: glial fibrillary acidic protein; I-ST: ipsilateral striatum; TSPO: 18 kDa translocator protein.

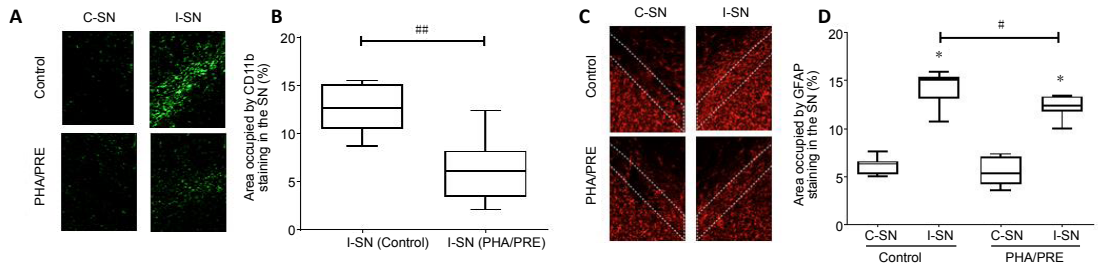

Figure 4 | **Neuroinflammation in the SN: CD11b and GFAP immunoreactivity.**
(A) Representative immunostaining of CD11b (green) in C-SN and I-SN in the control and PHA/PRE groups. (B) Quantification of the expression of CD11b (% area occupied) in I-SN. (C) Representative immunostaining of GFAP (red) in C-SN and I-SN in both groups. The white dotted line represents the ROI were GFAP expression was quantified. (D) Quantification of the expression of GFAP (% area occupied) in C-SN and I-SN. Data are represented as median ± SD. $n = 7$/group. *$P < 0.05$, *vs*. contralateral area in the same group (Wilcoxon test). #$P < 0.05$ and ##$P < 0.01$ (Mann-Whitney *U* test). C-SN: Controlateral SN; GFAP: glial fibrillary acidic protein; I-SN: ipsilateral SN; SN: substantia nigra.

Discussion

Several experimental findings from animal models of neurodegenerative diseases have demonstrated that partial neuroprotective effects can be obtained through the activation of either α7-nAChR or σ1-R (Liu et al., 2012; Stuckenholz et al., 2013; Suzuki et al., 2013; Bordia et al., 2015; Sérrière et al., 2015; Maurice, 2016; Foucault-Fruchard et al., 2017; Francardo et al., 2019). As it was suggested in a mouse model of AD that the interaction between these receptors could reinforce the beneficial effects (Maurice, 2016), we assessed here the consequences of the dual administration of agonists of these two therapeutic targets on neuron survival and glial activation in a rat model of early PD. We chose the α7-nAChR agonist PHA 543613 as this compound has already demonstrated neuroprotective and anti-inflammatory properties in rodent models of intracerebral hemorrhage and neurodegenerative disorders including AD and Huntington's disease (Krafft et al., 2012, 2013, 2017; Sadigh-Eteghad et al., 2015; Foucault-Fruchard et al., 2017, 2018). We also recently showed in a 6-OHDA rat model of PD that the administration of PHA 543613 provided partial protection of DA neuron endings associated with a reduction of microglial activation in the striatum (Sérrière et al., 2015). In the present study we therefore used this same α7-nAChR agonist, which has rapid brain penetration and high oral bioavailability (> 60%) in rats (Wishka et al., 2006; Acker et al., 2008), and administered it per os daily, from the day of 6-OHDA lesion until 14 days post lesion. The lesioned rats received concomitant administration of the σ1-R agonist PRE-084, a drug already used in animal studies including PD models (Francardo et al., 2014). We used this compound at a dose of 1 mg/kg as previously described (Francardo et al., 2014; Maurice 2016).

Our data has shown that, although no effect was observed in the amphetamine-induced rotation test, the dual PHA/PRE treatment induced partial (15–20%) protection of DA neurons, assessed by the DAT density in the striatum and immunoreactive TH in the SN. This effect was a slightly higher than the one we observed previously in the striatum using only PHA 543613 (Sérrière et al., 2015), although the assessment method was different (*in vivo* PET imaging *vs.* autoradiography in the present study). In a closely related rat model, similar neuroprotection was obtained using another α7-nAChR agonist ABT-107 (Bordia et al., 2015). Indeed in this study, the effect was associated with a partial recovery of motor deficits assessed by behavioral tests such as forepaw placement and adjusted stepping which may however be more sensitive than the classical induced-rotation test we used here (Meredith and Kang, 2006). The protection of DA neurons that we obtained also appeared quite similar to the results observed both in the striatum and SN with only PRE-084 treatment in a 6-OHDA-induced mouse model of PD (Francardo et al., 2014). In addition, the latter study showed that the neuroprotective effect was dependent on the activation of σ1-R and could be related to an upregulation of the neurotrophic factors, such as brain-derived neurotrophic factor and/or glial cell line-derived neurotrophic factor and their signaling pathways. Besides the neuronal effects, our dual PHA/PRE treatment reduced the glial activation consecutive to the 6-OHDA lesion, assessed by the TSPO density and GFAP staining in the striatum, and the CD11b and GFAP staining in the SN. In the lesioned striatum, the increased autoradiographic signal measured with the TSPO radioligand [^{3}H]PK-11195 indicated that microglial activation occurred (Papadopoulos et al., 2006; Chen and Guilarte 2008), concomitant to the loss of DA nerve endings measured with the DAT radioligand [^{125}I]PE2I, in agreement with previous results (Maia et al., 2012; Vetel et al., 2019). This microglial activation was accompanied with the astrogliosis already observed in this animal model (Vetel et al. 2020). We thus showed in the present study that these neuroinflammation biomarkers were reduced by the PHA/PRE treatment, not only in the striatum but also in the SN. Similar effects have already been observed in 6-OHDA PD rodent models after a single treatment with either an α7-nAChR or a σ1-R agonist (Suzuki et al., 2013; Francardo et al., 2014, 2019; Sérrière et al., 2015). In all these studies as in the present work, the expression of glial activation markers was attenuated but not totally inhibited, in agreement with the partial neuroprotective effects observed on DA neurons. One limitation of our study is that we did not include experimental groups of animals receiving exclusively the α7-nAChR or σ1-R agonists, thus preventing us from strictly comparing the impacts of a dual versus single agonist treatment. However, in relation to the results we previously obtained in a similar PD rat model using PHA 543613 alone at the same dose as here (Sérrière et al., 2015), the present findings suggest that a marked synergistic effect between these two drugs does not seem to occur but cannot be excluded. In this context, it would be of interest to test whether a lower dose of this same α7-nAChR agonist would have an effect not alone but in combination with the σ1-R agonist, in order to evaluate the potential additive effect of both compounds. Such a synergistic effect had previously been obtained using PRE-084 and the acetylcholinesterase inhibitor Donezepil together, but not using the combined PRE-084 and the N-methyl-D-aspartate receptor antagonist memantine, in a beta-amyloid-intracerebral administration mouse model of AD (Maurice, 2016). The latter data showed that the synergistic effect was specifically related to an interplay between σ1-R and α7-nAChR (Maurice, 2016). Using a different pharmacological approach in the transgenic 5XFAD mouse model of AD, no additional effects on the beta-amyloid load and behavioral deficits were obtained by associating scyllo-inositol with a non-steroidal anti-inflammatory drug (Aytan et al., 2013).

Various pharmacological combinations have already been proposed as potential PD therapy such as acamprosate, which targets glutamatergic, gamma-aminobutyric acid and DA neurotransmission, associated with the gamma-aminobutyric acid B receptor agonist baclofen. This approach led to a significant improvement of the motor dysfunction induced by 6-OHDA lesion in rats, but only to partial and modest DA neurons preservation (Hajj et al., 2015). In this context, multi-targeted drugs such as a monoamine oxidase (MAO) inhibitor plus an anti-cholinesterase drug or a monoamine oxidase inhibitor plus an adenosine A2 receptor inhibitor could represent a promising innovative approach, but their efficacy remains to be assessed (Mathew et al., 2019) .

The present study shows for the first time that the concomitant activation of α7-nAChR and σ1-R induces a protection of the nigro-striatal DA neurons and a concomitant reduction of microglia and astrocyte activation in a 6-OHDA-induced rat model of early PD. These findings strongly highlight the clear link between neurodegeneration and neuroinflammation. Nonetheless, these effects are limited, although potential interactions may occur between the targeted receptors. One restriction of this study is the choice of an animal model that does not express all the features of PD, in particular abnormal α-syuclein aggregation, and it could be worthwhile to assess such a pharmacological combination in a rodent model including this aspect of PD.

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