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7-Nitroindazole reduces L-DOPA-induced dyskinesias in non-human Parkinsonian primate

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Nitric oxide (NO) plays a pivotal role in integrating dopamine transmission in
the basal ganglia and has been implicated in the pathogenesis of Parkinson
disease (PD). The objective of this study was to ascertain whether the
NO synthase inhibitor, 7-nitroindazole (7-NI), is able to reduce L-DOPA-
induced dyskinesias (LIDs) in a non-human primate model of PD chronically
intoxicated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Six
Parkinsonian macaques were treated daily with L-DOPA for 3–4 months
until they developed LIDs. Three animals were then co-treated with a single
dose of 7-NI administered 45 min before each L-DOPA treatment. Dyskinetic
MPTP-treated monkeys showed a significant decrease in LIDs compared with
their scores without 7-NI treatment ($p < 0.05$). The anti-Parkinsonian effect of
L-DOPA was similar in all three monkeys with and without 7-NI co-treatment.
This improvement was significant with respect to the intensity and duration of
LIDs while the beneficial effect of L-DOPA treatment was maintained and
could represent a promising therapy to improve the quality of life of PD patients.

1. Introduction

While L-DOPA is the most effective treatment for Parkinson's disease (PD), its
chronic administration leads to motor complications expressed as fluctuations
in clinical responses and the appearance of abnormal involuntary movements
(AIMs), known as L-DOPA-induced dyskinesias (LIDs) [1]. Effective treat-
ment for LIDs is limited because, although molecular-based research has
suggested a potential role for several neurotransmitters and receptors [2–4],
the mechanisms underlying this phenomenon are still unclear [5–7]. Dopa-
minergic stimulation of the denervated striatum is a key mechanism
underlying LIDs [8]. It can exacerbate the sensitization process [9], induce acti-
vation pathways that provoke post-synaptic plastic changes in basal ganglia
circuits and facilitate AIMs [10], probably due to corticostriatal dendritic
spine alterations [11].

Besides dopaminergic mechanisms, alterations in several non-dopaminergic
systems have been linked to LIDs pathophysiology [12,13]. Significantly
enhanced glutamatergic neurotransmission has been found in the basal gang-
lial-thalamo-cortical circuit [14,15] and excessive amounts of N-methyl-

D-aspartate (NMDA) receptor are present in the striatum of Parkinsonian patients and animals with LIDs [16]. Thus, amantadine, non-competitive antagonist of NMDA receptor, has been used to the treatment of LIDs in Parkinson's disease patients [17,18]. However, it has been shown that this drug produces undesirable side effects in experimental and clinical models [18–20].

A growing body of evidence suggests that nitric oxide (NO) plays a role in the maintenance of LIDs [21–26], since it is synthesized in striatal interneurons by NO synthase (NOS) [27–29]. NO modulates the striatal function, changing its input–output relationship and producing a functionally significant impact on target neurons [30–33]. It has been suggested that (i) NO synthesis increases in the basal ganglia in experimental parkinsonism and in chronic treatment with L-DOPA [34–37], and (ii) nitrate concentrations and nitrite levels are high in the cerebrospinal fluid of patients with Parkinson's disease receiving dopamine replacement [38] and in patients with PD and LIDs [39]. Importantly, a potent NOS inhibitor, 7-nitroindazole (7-NI), has been successfully tested in dyskinetic rodents showing reduced AIMs [21–25,32,40–46].

Despite the large number of studies carried out in rodents, as far as we know there are no studies performed in non-human primates. Although the study in rodents is relevant, it must be taken into account that dyskinesias in non-human primates are remarkably similar to those seen in humans and the genetic and anatomo-physiological similarities with humans are greater than those of other rodent models such as rats and mice. Therefore, our aim was to determine whether the administration of 7-NI could be an efficient and safe treatment for reducing LIDs in non-human primates without affecting the therapeutic effect of L-DOPA.

2. Materials and methods

2.1. Non-human primate model of Parkinson disease

Monkeys were supervised by veterinarians and technicians skilled in the healthcare and maintenance of non-human primates. The animals were housed in primate cages under controlled conditions of humidity, light and temperature, and food (Masuri primate diet; Scientific Dietary Services, UK), fresh fruit and water were available *ad libitum*. Experiments were performed with six adult male cynomolgus monkeys (*Macaca fascicularis*, 3.8–4.5 kg) (purchased from R.C. Hartelust BV, The Netherlands) that were rendered Parkinsonian with methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-hydrochloride (Sigma, 0.3-0-4 mg kg^{-1} i.v. for a maximum period of 6 months, one injection every 2 weeks, $n = 6$) as previously described [47] (table 1). After reaching a stable parkinsonism (SP), monkeys were treated daily with Madopar [48] (Roche, 100 mg kg^{-1} L-DOPA and 25 mg kg^{-1} benserazide; ratio 4 : 1, $n = 6$) (termed L-DOPA hereafter) for 4 months until they developed stable and moderate–severe LIDs. 25 mg kg^{-1} of the NOS inhibitor (7-NI; Sigma-Aldrich) was dissolved in peanut oil solution and was administered subcutaneously every day 45 min prior to the injection of L-DOPA for 25 additional days ($n = 3$, randomly chosen).

Table 1. Experimental details of MPTP intoxication. Number and total dose of MPTP injections (in mg) and disability score before MPTP intoxication.

monkey	MPTP injections (number)	total dose (mg of MPTP)	disability score before
1	15	16.15	0
2	15	18.74	0
3	15	20.29	0
4	15	26.02	0
5	15	28.55	0
6	15	23.37	0

2.2. Behavioural assessments and clinical scales

The level of parkinsonism was assessed with a previously described [49,50] motor scale which evaluates the following symptoms: akinesia/bradykinesia, freezing, tremor, self-feeding, posture and spontaneous activity (maximum disability score, 25). Parkinsonian disability was assessed at the end of each session so as not to interfere with the assessment of levels of general activity. All monkeys reached similar SP levels. The intensity of LIDs was rated for each body segment (face, neck, trunk, arms and legs) every 30 min using a Dyskinesia Disability Scale (maximal score of 21 points) [51]. The dyskinetic score obtained was the sum of the scores for all body segments. LIDs were mainly choreic but dystonia was also observed. Stereotypies or licking were not considered as LIDs. The animals were placed in special observation cages for filming and the dyskinetic score was evaluated blindly with and without 7-NI co-administration. Motor evaluation was performed in the following endpoints: MPTP group when the six months of intoxication were finished, MPTP + L-DOPA group when the four months of treatment were finished and the group MPTP + L-DOPA + 7NI when the 25 days of 7NI treatment was completed.

2.3. Statistical analysis

Comparisons were performed using one-factor ANOVA with repeated measures followed by Newman–Keuls *post hoc* analysis. The area under the curve (AUC) [52], derived from the time-course experiments, was analysed by unpaired *t*-test. All data were expressed as mean group value ± s.e.m. A probability level of 5% ($p < 0.05$) or 1% ($p < 0.01$) was considered significant or very significant, respectively.

3. Results

We assessed the ability of 7-NI to change L-DOPA-induced diskinesia. Our study demonstrated that MPTP-treated monkeys developed moderate–severe LIDs when they are treated for 4 months with L-DOPA. The anti-Parkinsonian effect of L-DOPA was similar in all six monkeys, whose disability motor scores compared with their stable Parkinsonian state improved significantly (figure 1*a,b,d*). Their dyskinetic profiles showed a similar maximum (peak dose) at 80–100 min, finishing at 190–200 min. 7-NI co-administration preserved
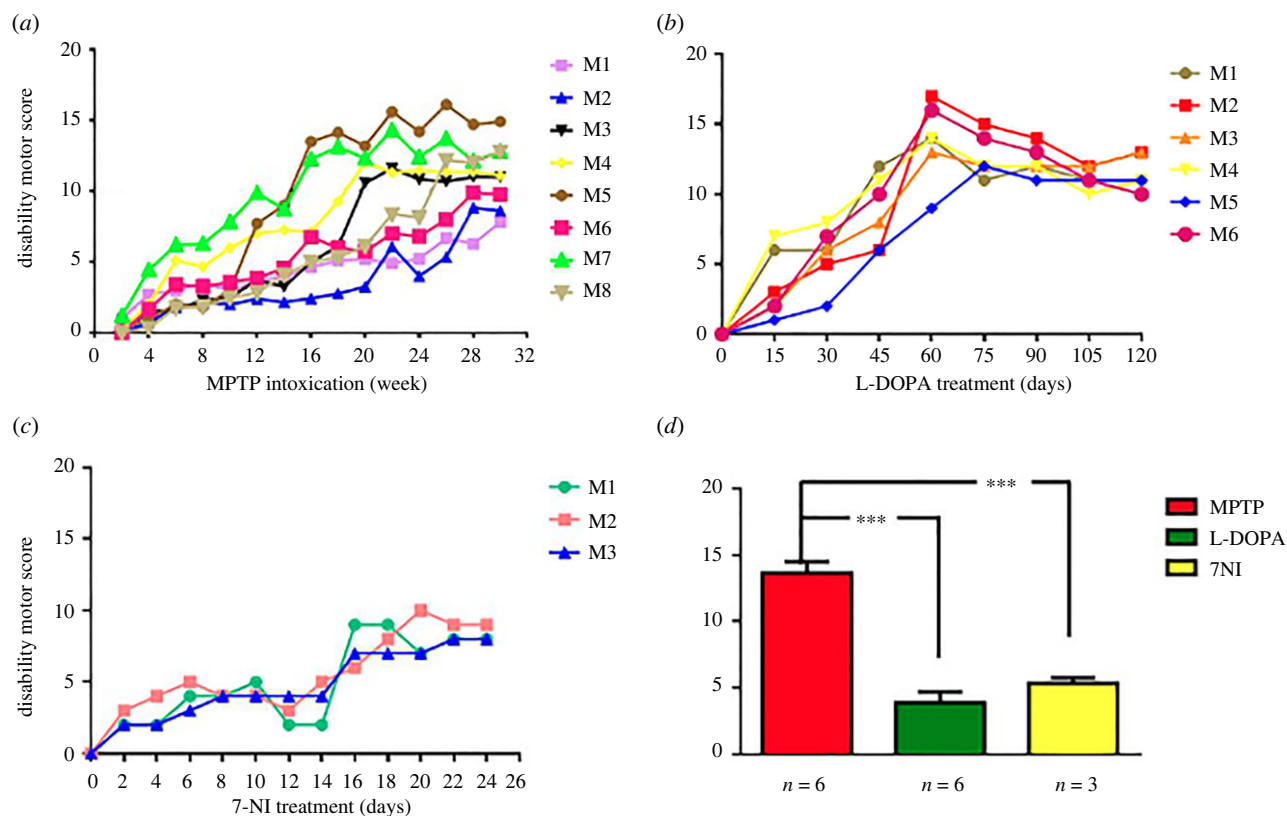
Figure 1. Disability motor score values in three different pharmacological states: (a) prior to L-DOPA treatment SP, (b) after L-DOPA treatment and (c) after L-DOPA + 7-NI co-administration. (d) Graph shows significant differences in disability motor score between SP data treated with MPTP compared with values observed after treatment of Parkinsonian mice with DOPA or L-DOPA + 7-NI co-administration; data are represented as mean ± s.e.m. $p < 0.001$ versus SP.

the beneficial anti-Parkinsonian effect of L-DOPA treatment without significant differences in the disability motor score ($p > 0.05$) (figure 1c,d). 7-NI dramatically decreased the intensity (figure 2b) and duration (figure 2c) of the LIDs, reducing the profile by more than 50%. Analyses of the time course and overall dyskinetic response (AUC) showed that 7-NI significantly reduced LIDs ($p < 0.001$) (figure 2d).

Monkey 1 received nine injections of MPTP over a period of four and half months. It developed significant bradykinesia, rigidity and freezing after the third MPTP injection. After the fifth MPTP injection resting tremor in upper limbs also appeared, and it showed a transient dystonia in the inferior limbs and in the oromandibular area, which disappeared after the eighth MPTP injection. After four months of L-DOPA treatment, stable body trunk LIDs, stereotypies in the hands, jerking motions and dystonic postures of lower limbs and tail developed. Fast choreic movements appeared 10 min after L-DOPA administration, lasting 200 min and reaching the peak dose at 110–120 min (intensity of 17/21). From the first co-administration with 7-NI, the dyskinetic profile changed, becoming significantly shorter (finishing at 130 min) ($p < 0.001$) (figure 2a,c) and reaching the peak dose at 60 min with a score of 6/21 ($p < 0.001$) (figure 2b,c).

Monkey 2 received nine injections of MPTP over four and half months. It developed rigidity, severe bradykinesia and slight tremor in upper limbs, and occasionally in the head. On several occasions following MPTP injection, abnormal movements of the mouth (repeated chewing) were observed. After four months of L-DOPA treatment, stable choreic movements of the whole body, but especially in the lower limbs appeared 10–15 min after L-DOPA administration lasting

200 min and reaching the highest peak dose at 120 min (intensity of 14/21) (figure 2a). From the first co-administration with 7-NI, the duration of LIDs was significantly shorter (finishing at 135 min) ($p < 0.001$) (figure 2a,c), reaching the peak dose at 60 min with a maximal score of 5/21 ($p < 0.001$) (figure 2b,c).

Monkey 3 received 15 injections of MPTP during a period of six months. Some vegetative symptoms were observed immediately after each dose but the animal returned to a normal state within 24 h. It showed rotational behaviour after the fourth MPTP injection. Bradykinesia, rigidity, freezing and tremor in the upper limbs, and oromandibular dystonia appeared after the seventh dose of MPTP. After four months of L-DOPA treatment stable choreic movements of all the body, especially fast in the lower limbs with the toe twisted, appeared 10–15 min after L-DOPA administration, lasting until 200 min and reaching the highest peak dose at 90 min (intensity of 14/21, figure 2a). From the first co-administration with 7-NI, the duration of LIDs was significantly shorter (finishing at 140 min) ($p < 0.001$) (figure 2a,c) reaching the peak dose at 60 min with a maximal score of 8/21 ($p < 0.001$) (figure 2b,c).

4. Discussion

Based on clinical data showing a direct correlation between the risk of LIDs development and the total amount of L-DOPA intake, a reduction in the daily doses of L-DOPA has been proposed by combining low doses of L-DOPA with non-dopaminergic therapies (for review, see [53]). In the present study, 7-NI, a NOS inhibitor, potently reversed
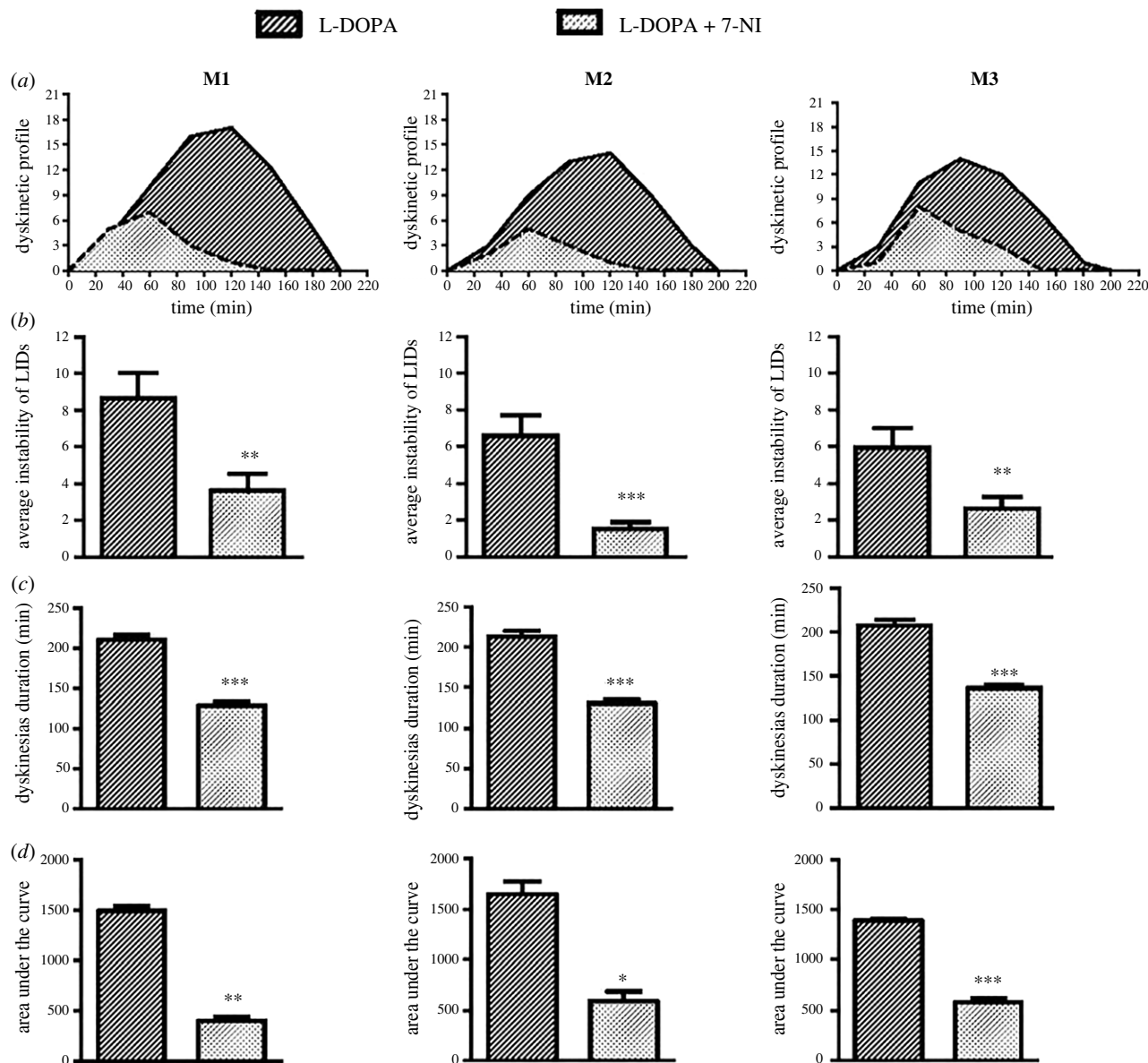
Figure 2. (a) Dyskinetic profile showing the intensity (dyskinesias score) and duration (min) in the three monkeys (M1, M2 and M3) with L-DOPA with and without 7-NI co-administration. (b) 7-NI significantly reduces the average intensity of LIDs over the time course: M1 without (9/21) and with (4/21) 7-NI co-treatment, M2 without (7/21) and with (1/21) 7-NI co-treatment, and M3 without (6/21) and with (3/21) 7-NI co-treatment (t-test, ** $p < 0.01$ and ***$p < 0.001$); and (c) duration of LIDs in MPTP-treated monkeys: M1 without (200 min) and with (130 min) 7-NI co-treatment, M2 without (200 min) and with (135 min) 7-NI co-treatment, and M3 without (200 min) and with (140 min) 7-NI co-treatment (t-test, ***$p < 0.001$). (d) The AUC, a measure that encompasses both intensity and duration of the LIDs, was significantly reduced with 7-NI co-administration for each monkey (paired t-test, ***$p < 0.001$ L-DOPA + 7-NI versus L-DOPA without 7-NI). These data are expressed as the mean AUC ± s.e.m. between 0 and 200 min in the three monkeys.

LIDs in monkeys. The antidyskinetic efficacy was not accompanied by detrimental effects on Parkinsonian motor symptoms. 7-NI effectively reduced the LIDs score but did not affect the disability motor score in MPTP-treated monkeys and injected with L-DOPA. Previous studies have demonstrated that the NOS inhibitor, 7-NI attenuated LIDs in 6-hidroxydopamine (6-OHDA) rats [21–25,32,40–43,45,46]. However, the present study provides the first evidence that 7-NI is effective against LIDs in MPTP-treated monkeys, which have been recognized as one of the best animal models to predict the clinical efficacy of compounds on LIDs (see [54,55] for a review).

Various measures of striatal NOS activity have indicated that NO signalling may be disrupted in patients with PD [56,57] and dopamine-depleted rats [58,59]. Studies by Del Bel et al. [21] in animals with an intact dopaminergic system, striatal NO—the enzyme soluble guanilate-cyclase

(sGC)—cyclicGM Phosphate transmission is likely to play a role in facilitating locomotor activity. Intrastriatal exposure to NOS and sGC inhibitors has been shown to depress basal locomotion and induce catalepsy [21,60]. However, we observed no cataleptic or lethargic effects in a motor evaluation of the monkeys. In fact, previous studies performed in baboons showed that 7-NI administration causes hyperactivity [61]. We observed no evidence of this in the 7-NI-treated-animals. Analysis of the AUC, which encompasses both the intensity and duration of the total dyskinetic response (figure 2d), demonstrates that the inhibition of NOS constantly and significantly reduces the LIDs profile (figure 2b,c). Pre-clinical studies aimed at decreasing NO signalling (and cGMP levels) have shown that co-administration of NOS inhibitors with L-DOPA attenuates LIDs [22,62]. NOS inhibition also improved the motor performance of the same animals on a rotarod test [22].

A possible mechanism to explain this phenomenon could be the role of striatal nitrergic interneurons, which are activated by both corticostriatal synaptic transmission (by direct synaptic contacts) and dopaminergic terminals (by D1/5 receptors) within striatal neural networks [33,63]. In fact, striatal NO plays a critical role in (i) activating cGMP in the medium spiny neurons (MSNs) upregulating cAMP [64] and inhibiting glutamate release in corticostriatal pathways [65], and/or (ii) provoking long-term depression in MSNs [33,66]. Therefore, we think it investigating the role of nitrergic pathways in LIDs using Parkinsonian monkeys would be of interest in future research.

Supported by many studies it has been proposed that abnormal neurotransmission and pathways not only dopamine may be involved in the pathophysiology of LIDs. Thus, recent paper has shown that a selective inhibitor of phosphodiesterase 1(PDE1) has potent antidyskinesic efficacy in non-human primate [67]. Further, several NMDA antagonists have shown efficacy against LIDs in chronically MPTP-treated monkeys [68], so that amantadine has been approved as treatment of LIDs [69]. However, amantadine has several adverse effects including hallucinations, constipation, dry mouth, peripheral oedema and nausea [70].

5. Opening up

There is interest to find new antidyskinesic candidates with the better safety profiles than amantadine. In this context, glutamate receptors are described to interact with NO, which may inhibit NMDA receptor by protein nitrosylation and destabilization of synaptic proteins [71]. Moreover, since 7-NI is able to reduce LIDs in Parkinsonian monkeys, different approaches could be evaluated using 7-NI co-administration. Many new selective neuronal NOS inhibitors are accessible, with reduced off-target effects related to other isoforms and promising pharmacokinetics to access brain tissues [72]. Additionally, Titze-de-Almeida *et al.* [73] described a siRNA which triggered a knockdown of nNOS mRNA and protein, reducing the cytotoxicity caused by 6-OHDA on SH-SY5Y cells. The intra-striatal injection of the siRNA-protected nigral dopaminergic neurons in 6-OHDA hemi-lesioned rats. Further on, in rodents 7-NI decreases dopamine turnover, (DOPAC/dopamine ratio) in the striatum of dyskinetic rats, which suggests the increase in the dopamine availability [41]. Ultimately, the combined treatment of amantadine and the NO inhibitor (7-NI), both in

low doses, may result in fewer side effects and a superior therapeutic benefit in LIDs [45].

6. Conclusion

Our results demonstrated that 7-NI is able to reduce the intensity and duration of LIDs without affecting anti-Parkinsonian benefits and could represent a promising therapy to improve the quality of life of PD patients.

References

1. Jankovic J, Stacy M. 2007 Medical management of levodopa-associated motor complications in patients with Parkinson's disease. *CNS Drugs* **21**, 677–692. (doi:10.2165/00023210-200721080-00005)

2. Herrero MT, Augood SJ, Asensi H, Hirsch EC, Agid Y, Obeso JA, Emson PC. 1996 Effects of L-DOPA-therapy on dopamine D2 receptor mRNA expression in the striatum of MPTP-intoxicated Parkinsonian monkeys. *Mol. Brain Res.* **42**, 149–155. (doi:10.1016/S0169-328X(96)00157-X)

3. Herrero MT *et al.* 1996 Consequence of nigrostriatal denervation and L-dopa therapy on the expression of glutamic acid decarboxylase messenger RNA in the pallidum. *Neurology* **47**, 219–224. (doi:10.1212/WNL.47.1.219)

4. Gottwald MD, Aminoff MJ. 2011 Therapies for dopaminergic-induced dyskinesias in Parkinson disease. *Ann. Neurol.* **69**, 919–927. (doi:10.1002/ana.22423)

5. Levy R, Vila M, Herrero MT, Faucheux B, Agid Y, Hirsch EC. 1995 Striatal expression of substance P and methionin-enkephalin in genes in patients with Parkinson's disease. *Neurosci. Lett.* **199**, 220–224. (doi:10.1016/0304-3940(95)12011-R)

6. Jenner P. 2008 Molecular mechanisms of L-DOPA-induced dyskinesia. *Nat. Rev. Neurosci.* **9**, 665–677. (doi:10.1038/nrn2471)

7. Cenci MA, Riggare S, Pahwa R, Eidelberg D, Hauser RA. 2020 Dyskinesia matters. *Mov. Disord.* **35**, 392–396. (doi:10.1002/mds.27959)

8. Olanow CW, Lees A, Obeso J. 2008 Levodopa therapy for Parkinson's disease: challenges and future prospects. *Mov. Disord.* **23**, S495–S496. (doi:10.1002/mds.22048)

9. Nadjar A, Gerfen CR, Bezard E. 2009 Priming for l-dopa-induced dyskinesia in Parkinson's disease: a

feature inherent to the treatment or the disease? *Prog. Neurobiol.* **87**, 1–9. (doi:10.1016/j.pneurobio.2008.09.013)

10. Cenci MA, Lindgren HS. 2007 Advances in understanding L-DOPA-induced dyskinesia. *Curr. Opin. Neurobiol.* **17**, 665–671. (doi:10.1016/j.conb.2008.01.004)

11. Scholz B *et al.* 2008 Striatal proteomic analysis suggests that first L-dopa dose equates to chronic exposure. *PLoS ONE* **3**, e1589. (doi:10.1371/journal.pone.0001589)

12. Heumann R, Moratalla R, Herrero MT, Chakrabarty K, Drucker-Colín R, Garcia-Montes JR, Simola N, Morelli M. 2014 Dyskinesia in Parkinson's disease: mechanisms and current non-pharmacological interventions. *J. Neurochem.* **130**, 472–489. (doi:10.1111/jnc.12751)

13. Cuenca L, Gil-Martinez AL, Cano-Fernandez L, Sanchez-Rodrigo C, Estrada C, Fernandez-Villalba E, Herrero MT. 2019 Parkinson's disease: a short story of 200 years. *Histol. Histopathol.* **34**, 573–591.

14. Sebastianutto I, Cenci MA. 2018 mGlu receptors in the treatment of Parkinson's disease and L-DOPA-induced dyskinesia. *Curr. Opin. Pharmacol.* **38**, 81–89. (doi:10.1016/j.coph.2018.03.003)

15. Guerra A, Suppa A, D'Onofrio V, Di Stasio F, Asci F, Fabbrini G, Berardelli A. 2019 Abnormal cortical facilitation and L-dopa-induced dyskinesia in Parkinson's disease. *Brain Stimul.* **12**, 1517–1525. (doi:10.1016/j.brs.2019.06.012)

16. Calon F, Rajput AH, Hornykiewicz O, Bédard PJ, Di Paolo T. 2003 Levodopa-induced motor complications are associated with alterations of glutamate receptors in Parkinson's disease. *Neurobiol. Dis.* **14**, 404–416. (doi:10.1016/j.nbd.2003.07.003)

17. Meissner WG *et al.* 2011 Priorities in Parkinson's disease research. *Nat. Rev. Drug Discov.* **10**, 377–393. (doi:10.1038/nrd3430)

18. Hubsher G, Haider M, Okun MS. 2012 Amantadine: the journey from fighting flu to treating Parkinson disease. *Neurology* **78**, 1096–1099. (doi:10.1212/WNL.0b013e31824e8f0d)

19. Crosby NJ, Deane KH, Clarke CE. 2003 Amantadine for dyskinesia in Parkinson's disease. *Cochrane Database Syst. Rev.* **2003**(2), CD003467.

20. Kastner A, Herrero MT, Hirsch EC, Guillen J, Luquin MR, Javoy-Agid F, Obeso JA, Agid Y. 1994 Decreased tyrosine hydroxylase content in the dopaminergic neurons of MPTP-intoxicated monkeys: effect of levodopa and GM1 ganglioside therapy. *Ann. Neurol.* **36**, 206–214. (doi:10.1002/ana.410360213)

21. Del Bel EA *et al.* 2005 Role of nitric oxide on motor behavior. *Cell Mol. Neurobiol.* **25**, 371–392. (doi:10.1007/s10571-005-3065-8)

22. Padovan-Neto FE, Echeverry MB, Tumas V, Del-Bel EA. 2009 Nitric oxide synthase inhibition attenuates l-DOPA-induced dyskinesias in a rodent model of Parkinson's disease. *Neuroscience* **159**, 927–935. (doi:10.1016/j.neuroscience.2009.01.034)

23. Padovan-Neto FE, Echeverry MB, Chiavegatto S, Del-Bel EA. 2011 Nitric oxide synthase inhibitor improves de novo and longterm l-DOPA-induced dyskinesia in hemiparkinsonian rats. *Front. Syst. Neurosci.* **10**, 40. (doi:10.3389/fnsys.2011.00040)

24. Takuma K, Tanaka T, Takahashi T, Hiramatsu N, Ota Y, Ago Y, Matsuda T. 2012 Neuronal nitric oxide synthase inhibition attenuates the development of L-DOPA-induced dyskinesiain hemi-Parkinsonian rats. *Eur. J. Pharmacol.* **683**, 166–173. (doi:10.1016/j.ejphar.2012.03.008)

25. Solís O, Espadas I, Del-Bel EA, Moratalla R. 2015 Nitric oxide synthase inhibition decreases l-DOPA-induced dyskinesia and the expression of striatal molecular markers in Pitx3(-/-) aphakia mice. *Neurobiol. Dis.* **73**, 49–59. (doi:10.1016/j.nbd.2014.09.010)

26. Jiménez-Jiménez FJ, Alonso-Navarro H, Herrero MT, García-Martín E, Agúndez JA. 2016 An update on the role of nitric oxide in the neurodegenerative processes of Parkinson's disease. *Curr. Med. Chem.* **23**, 2666–2679. (doi:10.2174/0929867323666160812151356)

27. Vincent SR, Kimura H. 1992 Histochemical mapping of nitric oxide synthase in the rat brain. *Neuroscience* **46**, 755–784. (doi:10.1016/0306-4522(92)90184-4)

28. Kiss JP, Vizi ES. 2001 Nitric oxide: a novel link between synaptic and nonsynaptic transmission. *Trends Neurosci.* **24**, 211–215. (doi:10.1016/S0166-2236(00)01745-8)

29. Hara MR, Snyder SH. 2007 Cell signaling and neuronal death. *Annu. Rev. Pharmacol. Toxicol.* **47**, 117–141. (doi:10.1146/annurev.pharmtox.47.120505.105311)

30. West AR, Grace AA. 2004 The nitric oxide-guanylyl cyclase signalling pathway modulates membrane activity states and electrophysiological properties of striatal medium spiny neurons recorded *in vivo*. *J. Neurosci.* **24**, 1924–1935. (doi:10.1523/JNEUROSCI.4470-03.2004)

31. Pierucci M, Galati S, Valentino M, Di Matteo V, Benigno A, Pitruzzella A, Muscat R, Di Giovanni G. 2011 Nitric oxide modulation of the basal ganglia circuitry: therapeutic implication for Parkinson's disease and other motor disorders. *CNS Neurol. Disord. Drug Targets* **10**, 777–791. (doi:10.2174/187152711798072329)

32. Del-Bel E, Padovan-Neto FE, Bortolanza M, Tumas V, Aguiar Jr AS, Raisman-Vozari R, Prediger RD. 2015 Nitric oxide, a new player in L-DOPA-induced dyskinesia? *Front. Biosci. (Elite Ed)* **7**, 168–192. (doi:10.2741/s431)

33. Shen W, Zhai S, Surmeier DJ. 2022 Striatal synaptic adaptations in Parkinson's disease. *Neurobiol. Dis.* **167**, 105686. (doi:10.1016/j.nbd.2022.105686)

34. Gomes MZ, Del Bel EA. 2003 Effects of electrolytic and 6-hydroxydopamine lesions of rat nigrostriatal pathway on nitric oxide synthase and nicotinamide adenine dinucleotide phosphate diaphorase. *Brain Res. Bull.* **62**, 107–115. (doi:10.1016/j.brainresbull.2003.08.010)

35. Gomes MZ, Raisman-Vozari R, Del Bel EA. 2008 A nitric oxide synthase inhibitor decreases 6-hydroxydopamine effects on tyrosine hydroxylase and neuronal nitric oxide synthase in the rat nigrostriatal pathway. *Brain Res.* **1203**, 160–169. (doi:10.1016/j.brainres.2008.01.088)

36. Chalimoniuk M, Langfort J. 2007 The effect of subchronic, intermittent L-DOPA treatment on neuronal nitric oxide synthase and soluble guanylyl cyclase expression and activity in the striatum and midbrain of normal and MPTP- treated mice. *Neurochem. Int.* **50**, 821–833. (doi:10.1016/j.neuint.2007.02.002)

37. Pavon N, Martin AB, Mendialdua A, Moratalla R. 2006 ERK phosphorylation and FosB expression are associated with L-DOPA-induced dyskinesia in hemiparkinsonian mice. *Biol. Psychiatry* **59**, 64–74. (doi:10.1016/j.biopsych.2005.05.044)

38. Molina JA, Jimenez-Jimenez FJ, Navarro JA, Vargas C, Gomez P, Benito- Leon J, Ortí-Pareja M, Cisneros E, Arenas J. 1996 Cerebrospinal fluid nitrate levels in patients with Parkinson's disease. *Acta Neurol. Scand.* **93**, 123–126. (doi:10.1111/j.1600-0404.1996.tb00186.x)

39. Santos-Lobato BL, Bortolanza M, Pinheiro LC, Batalhão ME, Pimentel ÂV, Capellari-Carnio E, Del-Bel EA, Tumas V. 2022 Levodopa-induced dyskinesias in Parkinson's disease increase cerebrospinal fluid nitric oxide metabolites' levels. *J. Neural Transm. (Vienna)* **129**, 55–63. (doi:10.1007/s00702-021-02447-4)

40. Del-Bel E, Padovan-Neto FE, Raisman-Vozari R, Lazzarini M. 2011 Role of nitric oxide in motor control: implications for Parkinson's disease pathophysiology and treatment. *Curr. Pharm. Des.* **17**, 471–488. (doi:10.2174/138161211795164176)

41. Del-Bel E, Padovan-Neto FE, Szawka RE, da-Silva CA, Raisman-Vozari R, Anselmo-Franci J, Romano-Dutra AC, Guimaraes FS. 2014 Counteraction by nitric oxide synthase inhibitor of neurochemical alterations of dopaminergic system in 6-OHDA-lesioned rats under L-DOPA treatment. *Neurotox Res.* **25**, 33–44. (doi:10.1007/s12640-013-9406-3)

42. Padovan-Neto FE, Ferreira NR, de Oliveira-Tavares D, de Aguiar D, da Silva CA, Raisman-Vozari R, Del Bel E. 2013 Anti-dyskinetic effect of the neuronal nitric oxide synthase inhibitor is linked to decrease of FosB/deltaFosB expression. *Neurosci. Lett.* **29**, 126–131. (doi:10.1016/j.neulet.2013.02.015)

43. Padovan-Neto FE, Cavalcanti-Kiwiatkoviski R, Carolino RO, Anselmo-Franci J, Del Bel EA. 2015 Effects of prolonged neuronal nitric oxide synthase inhibition on the development and expression of L-DOPA-induced dyskinesia in 6-OHDA-lesioned rats. *Neuropharmacology* **89**, 87–99. (doi:10.1016/j.neuropharm.2014.08.019)

44. Yuste JE, Echeverry MB, Ros-Bernal F, Gomez A, Ros CM, Campuzano CM, Fernandez-Villalba E, Herrero MT. 2012 7-Nitroindazole down-regulates dopamine/DARPP-32 signaling in neostriatal neurons in a rat model of Parkinson's disease. *Neuropharmacology* **63**, 1258–1267. (doi:10.1016/j.neuropharm.2012.07.031)

45. Bortolanza M, Bariotto-Dos-Santos KD, Dos-Santos-Pereira M, da-Silva CA, Del-Bel E. 2016 Antidyskinetic effect of 7-nitroindazole and sodium nitroprusside associated with amantadine in a rat

model of Parkinson's disease. *Neurotox Res.* **30**, 88–100. (doi:10.1007/s12640-016-9618-4)

46. Bortolanza M, Cavalcanti-Kiwiatkoski R, Padovan-Neto FE, da-Silva CA, Mitkovski M, Raisman-Vozari R, Del-Bel EA. 2015 Glial activation is associated with L-DOPA induced dyskinesia and blocked by a nitric oxide synthase inhibitor in a rat model of Parkinson's disease. *Neurobiol. Dis.* **73**, 377–387. (doi:10.1016/j.nbd.2014.10.017)

47. Barcia C, Sánchez Bahillo A, Fernández-Villalba E, Bautista V, Poza Y, Poza M, Fernández-Barreiro A, Hirsch EC, Herrero MT. 2004 Evidence of active microglia in substantia nigra pars compacta of Parkinsonian monkeys 1 year after MPTP exposure. *Glia* **46**, 402–409. (doi:10.1002/glia.20015)

48. Ouattara B, Hoyer D, Grégoire L, Morissette M, Gasparini F, Gomez-Mancilla B, Di Paolo T. 2010 Changes of AMPA receptors in MPTP monkeys with levodopa-induced dyskinesias. *Neuroscience* **167**, 1160–1167. (doi:10.1016/j.neuroscience.2010.03.022)

49. Herrero MT, Hirsch EC, Kastner A, Ruberg M, Luquin MR, Laguna J, Javoy-Agid F, Obeso JA, Agid Y. 1993 Does neuromelanin contribute to the vulnerability of catecholaminergic neurons in monkeys intoxicated with MPTP? *Neuroscience* **56**, 499–511. (doi:10.1016/0306-4522(93)90349-K)

50. Barcia C, Bautista V, Sánchez-Bahillo A, Fernández-Villalba E, Navarro-Ruis JM, Barreiro AF, Poza Y, Poza M, Herrero MT. 2003 Circadian determinations of cortisol, prolactin and melatonin in chronic methyl-phenyl-tetrahydropyridine-treated monkeys. *Neuroendocrinology* **78**, 118–128. (doi:10.1159/000071967)

51. Hadj Tahar A, Grégoire L, Darré A, Bélanger N, Meltzer L, Bédard PJ. 2004 Effect of a selective glutamate antagonist on L-DOPA-induced dyskinesias in drug-naive Parkinsonian monkeys. *Neurobiol. Dis.* **15**, 171–176. (doi:10.1016/j.nbd.2003.10.007)

52. Berton O, Guigoni C, Li Q, Bioulac BH, Aubert I, Gross CE, Dileone RJ, Nestler EJ, Bezard E. 2009 Striatal overexpression of DeltaJunD resets L-DOPA-induced dyskinesia in a primate model of Parkinson disease. *Biol. Psychiatry* **66**, 554–561. (doi:10.1016/j.biopsych.2009.04.005)

53. Fabbrini A, Guerra A. 2021 Pathophysiological mechanisms and experimental pharmacotherapy for L-dopa-induced dyskinesia. *J. Exp. Pharmacol.* **29**, 469–485. (doi:10.2147/JEP.S265282)

54. Emborg ME. 2007 Nonhuman primate models of Parkinson's disease. *ILAR J.* **48**, 339–355. (doi:10.1093/ilar.48.4.339)

55. Morissette M, Di Paolo T. 2018 Non-human primate models of PD to test novel therapies. *J. Neural Transm.* **125**, 291–324. (doi:10.1007/s00702-017-1722-y)

56. Bockelmann R, Wolf G, Ransmayr G, Riederer P. 1994 NADPH-diaphorase/nitric oxide synthase containing neurons in normal and Parkinson's disease putamen. *J. Neural Transm.* **7**, 115–121. (doi:10.1007/BF02260966)

57. Eve DJ, Nisbet AP, Kingsbury AE, Hewson EL, Daniel SE, Lees AJ, Marsden CD, Foster OJ. 1998 Basal ganglia neuronal nitric oxide synthase mRNA expression in Parkinson's disease. *Mol. Brain Res.* **63**, 62–71. (doi:10.1016/S0169-328X(98)00259-9)

58. De Vente J, Markerink-van Ittersum M, Van Abeelen J, Emson PC, Axer H, Steinbusch HWM. 2000 NO-mediated cGMP synthesis in cholinergic neurons in the rat forebrain: effects of lesioning dopaminergic or serotonergic pathways on nNOS and cGMP synthesis. *Eur. J. Neurosci.* **12**, 507–519. (doi:10.1046/j.1460-9568.2000.00927.x)

59. Sancesario G, Giorgi M, D'Angelo V, Modica A, Martorana A, Morello M, Bengtson CP, Bernardi G. 2004 Down-regulation of nitrergic transmission in the rat striatum after chronic nigrostriatal deafferentation. *Eur. J. Neurosci.* **20**, 989–1000. (doi:10.1111/j.1460-9568.2004.03566.x)

60. Echeverry MB, Salgado ML, Ferreira FR, da-Silva CA, Del Bel EA. 2007 Intracerebroventricular administration of nitric oxide-sensitive guanylyl cyclase inhibitors induces catalepsy in mice. *Psychopharmacology (Berl)* **194**, 271–278. (doi:10.1007/s00213-007-0834-8)

61. Hantraye P, Brouillet E, Ferrante R, Palfi S, Dolan R, Matthews RT, Beal MF. 1996 Inhibition of neuronal nitric oxide synthase prevents MPTP- induced Parkinsonism in baboons. *Nat. Med.* **2**, 1017–1021. (doi:10.1038/nm0996-1017)

62. Bariotto-Dos-Santos K, Padovan-Neto FE, Bortolanza M, Dos-Santos-Pereira M, Raisman-Vozari R, Tumas V, Del Bel E. 2019 Repurposing an established drug: an emerging role for methylene blue in L-DOPA-induced dyskinesia. *Eur. J. Neurosci.* **49**, 869–882. (doi:10.1111/ejn.14079)

63. West AR, Galloway MP, Grace AA. 2002 Regulation of striatal dopamine neurotransmission by nitric oxide: effector pathways and signaling mechanisms. *Synapse* **44**, 227–245. (doi:10.1002/syn.10076)

64. Lin DT, Fretier P, Jiang C, Vincent SR. 2010 Nitric oxide signaling via cGMP-stimulated phosphodiesterase in striatal neurons. *Synapse Jun.* **64**, 460–466. (doi:10.1002/syn.20750)

65. Calabresi P, Gubellini P, Centonze D, Sancesario G, Morello M, Giorgi M, Pisani A, Bernardi GA. 1999 Critical role of the nitric oxide/cGMP pathway in corticostriatal long-term depression. *J. Neurosci.* **19**, 2489–2499. (doi:10.1523/JNEUROSCI.19-07-02489.1999)

66. West R, Tseng K. 2011 Nitric oxide-soluble guanylyl cyclase-cyclic GMP signalling in the striatum: new targets for the treatment of Parkinson's disease? *Front. Syst. Neurosci.* **5**, 1–9. (doi:10.3389/fnsys.2011.00055)

67. Enomoto T, Nakako T, Goda M, Wada E, Kitamura A, Fujii Y, Ikeda KA. 2021 Novel phosphodiesterase 1 inhibitor reverses L-dopa-induced dyskinesia, but not motivation deficits, in monkeys. *Pharmacol. Biochem. Behav.* **205**, 173183. (doi:10.1016/j.pbb.2021.173183)

68. Fox SH, Lang AE, Brotchie JM. 2006 Translation of nondopaminergic treatments for levodopa-induced dyskinesia from MPTP-lesioned nonhuman primates to phase IIa clinical studies: keys to success and roads to failure. *Mov. Disord.* **21**, 1578–1594. (doi:10.1002/mds.20936)

69. Elmer LW, Juncos JL, Singer C, Truong DD, Criswell SR, Parashos S, Felt L, Johnson R, Patni R. 2018 Pooled analyses of phase III studies of ADS-5102 (amantadine) extended-release capsules for dyskinesia in Parkinson's disease. *CNS Drugs* **32**, 399–400. (doi:10.1007/s40263-018-0510-z)

70. Sharma VD, Lyons KE, Pahwa R. 2018 Amantadine extended-release capsules for levodopa-induced dyskinesia in patients with Parkinson's disease. *Ther. Clin. Risk Manag.* **14**, 665–673. (doi:10.2147/TCRM.S144481)

71. Kopincová J, Púzserová A, Bernátová I. 2011 Biochemical aspects of nitric oxide synthase feedback regulation by nitric oxide. *Interdiscip. Toxicol.* **4**, 63–68. (doi:10.2478/v10102-011-0012-z)

72. Pensa AV, Cinelli MA, Li H, Chreifi G, Mukherjee P, Roman LJ, Martásek P, Poulos TL, Silverman RB. 2017 Hydrophilic, potent, and selective 7-substituted 2-aminoquinolines as improved human neuronal nitric oxide synthase inhibitors. *J. Med. Chem.* **60**, 7146–7165. (doi:10.1021/acs.jmedchem.7b00835)

73. Titze-de-Almeida R, Titze-de-Almeida SS, Ferreira NR, Fontanari C, Faccioli LH, Del Bel E. 2019 Suppressing nNOS enzyme by small-interfering RNAs protects SH-SY5Y Cells and nigral dopaminergic neurons from 6-OHDA injury. *Neurotox Res.* **36**, 117–131. (doi:10.1007/s12640-019-00043-9)