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REVIEW ARTICLE

Managing Parkinson's disease: moving ON with NOP

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The opioid-like neuropeptide nociceptin/orphanin FQ (N/OFQ) and its receptor (NOP receptor) contribute to Parkinson's disease (PD) and motor complications associated with levodopa therapy. The N/OFQ–NOP receptor system is expressed in cortical and subcortical motor areas and, notably, in dopaminergic neurons of the substantia nigra compacta. Dopamine depletion, as in rodent models of PD results in up-regulation of N/OFQ transmission in the substantia nigra and down-regulation of N/OFQ transmission in the striatum. Consistent with this, NOP receptor antagonists relieve motor deficits in PD models by reinstating the physiological balance between excitatory and inhibitory inputs impinging on nigro-thalamic GABAergic neurons. NOP receptor antagonists also counteract the degeneration of nigrostriatal dopaminergic neurons, possibly by attenuating the excitotoxicity or modulating the immune response. Conversely, NOP receptor agonists attenuate levodopa-induced dyskinesia by attenuating the hyperactivation of striatal D_1 receptor signalling in neurons of the direct striatonigral pathway. The N/OFQ–NOP receptor system might represent a novel target in the therapy of PD.

1 | PHARMACOLOGY OF THE NOCICEPTIN/ORPHANIN FQ-NOCICEPTIN OPIOID PEPTIDE RECEPTOR SYSTEM

The nociceptin opioid peptide (**NOP) receptor**, a GPCR and the fourth member of the **opioid receptor** family, was discovered in 1994, after cloning of the other three opioid receptors **μ**, **δ**, and **κ** (Mollereau et al., 1994). Soon thereafter, a heptadecapeptide (FGGFTGARKSARKLANQ), independently isolated by two laboratories from rat and porcine brains, was identified as the endogenous agonist ligand for the NOP receptor and named nociceptin and orphanin FQ, respectively (Meunier et al., 1995; Reinscheid et al., 1995). This endogenous peptide is now commonly referred to as **nociceptin/orphanin FQ** (N/OFQ). N/OFQ is similar in structure and sequence to the other endogenous opioid peptides, particularly the κ opioid peptide dynorphin. However, it does not bind to the classical opioid receptors μ, δ, and κ (Gintzler, Adapa, Toll, Medina, & Wang, 1997). Conversely, the endogenous opioid peptides have no affinity for the NOP receptor (Bunzow et al., 1994; Mollereau et al., 1994; Wang et al., 1994). Like the other opioid receptors, however, the NOP receptor is also a $G_{i/o}$-protein-coupled receptor, and activation of the receptor by N/OFQ or other agonists results in inhibition of **AC** and **cAMP** production, increase in K^+ conductance, and inhibition of Ca^{+2} conductance (Hawes, Graziano, & Lambert, 2000). Overall, activation of the NOP receptor inhibits cellular neuronal activity, leading to a reduction in the release of neurotransmitters such as **dopamine**, **glutamate**, **GABA**, and **ACh** by presynaptically located NOP receptors or inhibiting neuronal excitability via postsynaptic NOP receptors (Schlicker & Morari, 2000; Yu, Fein, Phan, Evans, & Xie, 1997).

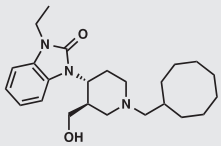
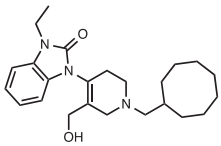
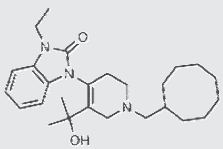
Abbreviations: BG, basal ganglia; LID, L-DOPA-induced dyskinesia; MPP^+, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MSNs, medium-sized spiny neurons; NMS, nonmotor symptoms; N/OFQ, nociceptin/orphanin FQ; NOP, nociceptin opioid peptide; PD, Parkinson's disease; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus; TTX, tetrodotoxin.

The NOP receptor and N/OFQ are widely expressed in the central and peripheral nervous system and tissues as well as the immune system and shown to modulate various biological functions such as locomotor activity, food intake, emotional states, pain transmission, learning and memory, cardiovascular functions, diuresis, gastrointestinal motility, and motivational states (Lambert, 2008; Toll, Bruchas, Calo, Cox, & Zaveri, 2016). Expression is similar among species with some notable exceptions such as prominent striatal N/OFQ–NOP receptor expression in non-human primates (Bridge, Wainwright, Reilly, & Oliver, 2003) and humans (Berthele et al., 2003), compared to rodents (Neal et al., 1999a; Neal et al., 1999b). The study of the functions of the NOP receptor in normal and pathological states has been significantly facilitated by the development of peptide and non-peptide NOP receptor ligands (Table 1). Below is a brief overview of some salient peptide and non-peptide NOP receptor agonists and antagonists frequently used in NOP research, including studies discussed in this article. The reader is referred to several excellent reviews on recent advances in the development of new NOP ligands (Gavioli & Calo, 2013; Mustazza, Pieretti, & Marzoli, 2018; Zaveri, 2016; Zaveri & Meyer, 2019).

1.1 | Peptide NOP receptor ligands

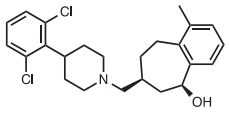
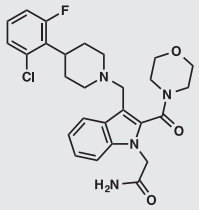
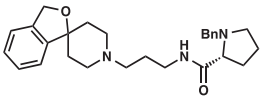
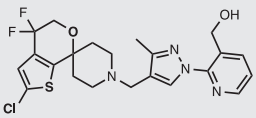
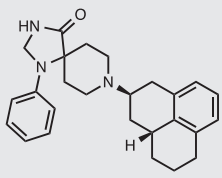
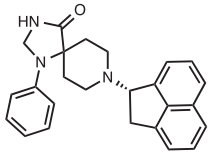
Structure–activity relationship studies of endogenous N/OFQ showed that the shortest truncated peptide sequence that retained full agonist activity was **N/OFQ (1-13)** (Dooley & Houghten, 1996; Reinscheid, Ardati, Monsma, & Civelli, 1996). Further peptide bond modifications of this shortened sequence resulted in the first NOP partial agonist ligand **[Phe^1(CH2-NH)Gly^2]N/OFQ(1-13**; Calo et al., 1998; Guerrini et al., 1998) and later to the first NOP antagonist peptide ligand **[Nphe^1]N/OFQ(1-13**; Calo et al., 2000). Subsequent modifications to

TABLE 1 Synopsis of the main pharmacological properties of NOP receptor ligands

		Binding affinity		Functional efficacy		
		NOP		GTPγS	α^a	pK_b/pA_2
Compound	Structure	pKi	Selectivity	pEC_{50}		
N/OFQ		9.91	>1,000	8.75	1	
N/OFQ (1-13)-NH_2		10.24	276	9.28	0.86	
NOP antagonists						
[Nphe^1]	[Nphe1]N/OFQ (1-13)-NH_2	8.39 (Ref. 1)	269		–	7.33 (Ref. 2)
UFP-101	[(Nphe)^1,Arg^{14},Lys^{15}]N/OFQ	10.24	>1,000		–	8.85 (Ref. 2)
J-113397		9.15	147			9.08 (Ref. 3)
Trap-101		8.36 (Ref. 3)	100			8.55 (Ref. 3)
GF-4		7.46 (Ref. 4)	5			7.27 (Ref. 4; note[a])
SB-612111		9.18 (Ref. 5)	>1,000		–	9.70 (Ref. 5)

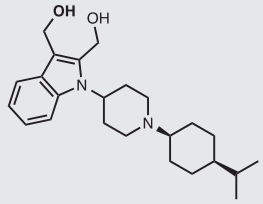
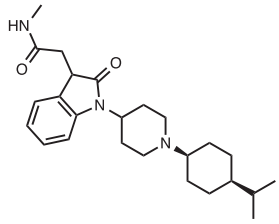
(Continues)

TABLE 1 (Continued)

Compound	Structure	Binding affinity NOP pKi	Selectivity	Functional efficacy GTPγS pEC_{50}	α^a	pK_b/pA_2
						
Nik-21273		8.10 (Ref. 6; see note[b])	>1,000			7.77 (Ref. 6; note[a])
C-24		9.62 (Ref. 7)	794		–	9.98 (Ref. 7)
LY2940094		9.95 (Ref. 8)	>1,000			9.77 (Ref. 8)
NOP agonists						
[F/G]	[F/G]N/OFQ (1-13)-NH_2	8.00 (Ref. 9)	67	8.05 (Ref. 10)	0.67	
[$Arg^{14}Lys^{15}$]N/OFQ		9.96 (Ref. 11)		8.96 (Ref. 11)	1.00	
UFP-102	[(pF)Phe^4,Arg^{14},Lys^{15}]N/OFQ-NH_2	10.67 (Ref. 12)	>200	10.12 (Ref. 12)	1.26	
UFP-112	[(pF)Phe^4,Aib^7,Arg^{14},Lys^{15}]N/OFQ	10.55 (Ref. 13)	>1,000	10.55 (Ref. 13)	1.03	
Ro 64-6198		9.41 (Ref. 14)	>100	8.09 (Ref. 2)	0.89	
Ro 65-6570		8.25 (Ref. 15)	10	7.73	1.01	

(Continues)

TABLE 1 (Continued)

Compound	Structure	Binding affinity NOP pKi	Selectivity	Functional efficacy GTPγS pEC_{50}	α^a	pK_b/pA_2
AT-390		9.05 (Ref. 16; note[b])	50	7.82	1.10	
AT-403		8.94 (Ref. 16; note[b])	90	8.20	1.05	

1. Calo et al., 2000; 2. McDonald et al., 2003; 3. Trapella et al., 2006; 4. Volta et al., 2010; 5. Spagnolo et al., 2007; 6. Marti et al., 2013; 7. Fischetti et al., 2009; 8. Toledo et al., 2014; 9. Varani et al., 1999; 10. Wright et al., 2003; 11. Okada et al., 2008; 12. Carra et al., 2005; 13. Arduin et al., 2007; 14. Jenck et al., 2000; 15. Hashiba et al., 2001; 16. Arcuri et al., 2018.

[a]The functional assay used to measure pA_2 was inhibition of N/OFQ-induced Ca mobilization performed in CHO cells stably expressing the NOP-$G\alpha_{qi5}$ chimeric protein that signals through Ca^{+2}.

[b]The pKi value was calculated from the reported Ki value.

the peptide sequence, particularly the introduction of the Arg^{14} and Lys^{15} residues into the N/OFQ sequence, resulted in high affinity, highly potent ligands, such as the NOP agonist **[Arg^{14}, Lys^{15}]N/OFQ** (Okada et al., 2000), which was shown to be 30-fold more potent than N/OFQ and elicit long-lasting effects in vivo (Rizzi et al., 2002). Further enhancements in affinity and potency were obtained by chemical modifications of other residues, resulting in some of the most potent, highly selective and long-lasting peptide agonists yet reported, [(pF)Phe^4,Arg^{14},Lys^{15}]N/OFQ (**UFP-102**; Bigoni et al., 2002; Rizzi et al., 2002) and [(pF)Phe^4,Aib^7,Arg^{14},Lys^{15}]N/OFQ (**UFP-112**; Rizzi et al., 2007). The [Arg^{14}, Lys^{15}] modification combined with the N-terminal [$Nphe^1$] "antagonist" modification in N/OFQ generated a potent antagonist peptide ligand [$(Nphe)^1$,Arg^{14},Lys^{15}]N/OFQ (**UFP-101**; Calo et al., 2002), which is one of the most widely used, highly selective, peptidic NOP antagonist ligand (Calo et al., 2005).

1.2 | Non-peptide NOP receptor ligands

One of the very first small-molecule NOP ligands reported was the NOP *antagonist*, **J-113397** (Kawamoto et al., 1999). J-113397 has nanomolar affinity for NOP receptors and modest selectivity versus the μ receptor but is a potent NOP antagonist (Ozaki et al., 2000). Other close structural analogues of J-113397 were subsequently reported and characterized in vivo, such as the achiral analogue **Trap-101** (Trapella et al., 2006) and the dimethylated Trap-101 analogue, GF-4 (Volta et al., 2010). The other widely used NOP antagonist tool compound **SB-612111**, reported by GSK, shows subnanomolar affinity for the NOP receptor and excellent selectivity versus the other opioid receptors (Zaratin et al., 2004), compared to J-113397. SB-612111 potently antagonizes the effects of N/OFQ in cellular functional assays in vitro with 3–10 times greater potency than J-113397 (Spagnolo et al., 2007) and in vivo, for example, on the orexigenic effect of N/OFQ (Rizzi et al., 2007). A related phenylpiperidine-based NOP antagonist, **Nik-21273**, was reported to be very selective for the NOP receptor but less potent than SB-612111 at native NOP receptors (Marti et al., 2013). A novel spiropiperidine NOP antagonist, **Compound 24** (C-24), reported by Banyu Pharma, was found to have excellent selectivity and subnanomolar affinity for the NOP receptor and potent in vivo activity in reversing the locomotor-suppressing effects of N/OFQ (Goto et al., 2006). A recent addition to the class of nonpeptide NOP ligands is **LY2940094**, reported by Eli Lilly (Toledo et al., 2014). LY2940094 (now called **BTRX-246040**) is orally active and shows excellent efficacy in animal models of depression (Post et al., 2016) and alcohol use disorders (Rorick-Kehn et al., 2016) and is under clinical investigation for these applications (Witkin, Wallace, &

Martin, 2019) and in Phase II clinical trials for Parkinson's disease (PD; NCT03608371, 2019). Among non-peptide NOP agonists, **Ro 64-6198**, the first small-molecule NOP agonist reported (Jenck et al., 2000) is also the most widely studied non-peptide NOP *agonist* to date (Zaveri, 2016). Ro 64-6198 has subnanomolar affinity for the NOP receptor, greater than 100-fold selectivity over the classical opioid receptors, and agonist potency comparable to that of N/OFQ itself (Dautzenberg et al., 2001). A close congener, **Ro 65-6570** has slightly lower affinity and selectivity than Ro 64-6198, but similar agonist potency (Hashiba et al., 2001; Rover et al., 2000). Newer NOP agonists such as AT-390 and **AT-403**, with chemical structures different from the Roche NOP agonists, have been reported to exhibit nano-to-subnanomolar affinity for NOP, >100-fold selectivity versus the opioid (particularly the μ) receptors and high agonist potency (see Table 1). These and several other NOP agonists shown in Table 1 were recently characterized as tool compounds in several in vivo pharmacological assays involving NOP function (Arcuri et al., 2018; Ferrari et al., 2017).

2 | EXPRESSION OF PREPRON/OFQ AND NOP IN THE PD BRAIN

The intense expression of the N/OFQ–NOP receptor system in rat mesencephalic dopaminergic areas (Neal et al., 1999a; Neal et al., 1999b) and the early findings that N/OFQ regulates the activity of the mesoaccumbal dopaminergic system in rats (Murphy, Ly, & Maidment, 1996; Murphy & Maidment, 1999) inspired the seminal in situ hybridization study of Watson and collaborators (Norton, Neal, Kumar, Akil, & Watson, 2002). IN this study, ~50% of tyrosine hydroxylase (TH)-positive cells in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) expressed NOP mRNA whereas the vast majority (~80%) of NOP-positive cells expressed TH. Conversely, a negligible percentage of TH-positive cells (<2%) express the N/OFQ precursor preproN/OFQ (ppN/OFQ) mRNA, which, instead, co-localizes with glutamic acid decarboxylase (GAD) in about half the neurons, suggesting that VTA/SNc dopaminergic neurons express the NOP receptor whereas N/OFQ is released by GABA-ergic interneurons. Another breakthrough of that study was the finding that destruction of mesencephalic dopaminergic neurons with the parkinsonian toxin 6-hydroxydopamine (6-OHDA; Schwarting & Huston, 1996a; Schwarting & Huston, 1996b) resulted in a dramatic increase of ppN/OFQ mRNA (but not of the percentage of ppN/OFQ-positive cells that expressed GAD) and reduction of NOP mRNA in both VTA and SNc, with larger changes observed in the SNc (Table 2). As the reduction of NOP mRNA was associated with a ~70% reduction of N/OFQ-stimulated [^{35}S]GTPγS binding, it was concluded that dopamine depletion, and more generally the parkinsonian condition, is characterized by an up-regulation of ppN/OFQ and down-regulation of NOP receptor expression in the midbrain dopaminergic regions (Norton et al., 2002). This finding was later confirmed by Morari and collaborators (Marti et al., 2005). Interestingly, this study also disclosed a similar but milder change in the substantia nigra reticulata (SNr; twofold increase of ppN/OFQ and ~25% loss of NOP receptor mRNA), suggesting that up-regulation of N/OFQ transmission also affects the activity of the nigro-thalamic pathway, that is, the nigral output. An increase in N/OFQ expression in SNc under dopamine-depleting conditions was consistently found in studies employing other parkinsonian toxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; Gouty et al., 2010), its active metabolite 1-methyl-4-phenylpyridinium (**MPP$^+$**; Di Benedetto et al., 2009), and also a combination of the herbicide **paraquat** and the fungicide maneb (Bastias-Candia et al., 2015). Thus, dysregulation of the N/OFQ–NOP system appears to be a common feature of the parkinsonian brain. Cox and collaborators (Gouty et al., 2010) further demonstrated that the elevation of ppN/OFQ specifically occurs in a subset of GABAergic neurons throughout the SNr. ppN/OFQ up-regulation and NOP receptor down-regulation are likely to be linked events but it is not known which occurs first. As NOP receptors are expressed on dopaminergic neurons, the up-regulation of N/OFQ in GABA neurons might be a compensatory response to the degeneration of dopaminergic cells. However, an increase of nigral ppN/OFQ in the absence of any changes of NOP mRNA was observed following partial (80%) nigral dopamine depletion (Di Benedetto et al., 2009), indicating that the increase of N/OFQ expression is likely to be the primary event. Opposite to that observed in SN, striatal dopamine depletion is associated with a reduction (~50%) of ppN/OFQ expression, as measured with RT-PCR (Di Benedetto et al., 2009). Further, increased N/OFQ binding was demonstrated by autoradiography in the dopamine-depleted striatum of 6-OHDA hemilesioned rats, indicating NOP receptor up-regulation in this brain region (Marti et al., 2012). Interestingly, chronic treatment with **L-DOPA** leading to dyskinesia did not change this pattern of NOP expression levels (Marti et al., 2012). These adaptive changes to dopamine depletion seem to specifically affect the nigrostriatal pathway because ppN/OFQ levels in other brain areas receiving dopaminergic innervation, such as cerebral cortex, nucleus accumbens, thalamus, and globus pallidus, were unaffected (Marti et al., 2010).

Preliminary analysis in humans yielded a conflicting picture. In fact, a reduction of ppN/OFQ mRNA and no change in NOP mRNA was found in the SN of PD patients (Collins et al., 2015). This discrepancy with preclinical data is not easily explained and may be due to the occurrence of compensatory mechanisms to prevent excessive NOP receptor activation or the existence of species-dependent regulatory mechanisms. In addition, it is also possible that the biochemistry of post-mortem human brains that usually reflects changes typical of very late stages of the disease might not correspond to the biochemical changes occurring in acute or subacute PD models.

3 | N/OFQ RELEASE IN PD

To prove that the increase of nigral ppN/OFQ mRNA observed after dopamine depletion is coupled with an increase of N/OFQ release and a corresponding elevation of N/OFQ levels in the extracellular space, in vivo microdialysis was performed and

TABLE 2 Synopsis of the studies on ppN/OFQ and NOP gene expression

Model	Species	Readout	Area	% Change	Extent of lesion	Reference
6-OHDA (mfb)	Rat	ppN/OFQ mRNA	SNc VTA	+200 +60	Full[a]	Norton et al., 2002
		NOP mRNA	SNc VTA	–85 –85		
6-OHDA (mfb)	Rat	ppN/OFQ mRNA	SNc SNr	+200 +100	Full[a]	Marti et al., 2005
		NOP mRNA	SNc SNr	–50 –20		
6-OHDA (i.c.v.)	Rat	ppN/OFQ mRNA	SN striatum	+50 –50	Intermediate[b]	Di Benedetto et al., 2009
		NOP mRNA	SN striatum	n.c. –50		
MPP^+ (i.c.v.)	Rat	ppN/OFQ mRNA	SN striatum	+60 –50	Intermediate[b]	
		NOP mRNA	SN striatum	–30 –50		
MPTP (systemic)	Mouse	ppN/OFQ mRNA	SNc SNr VTA striatum STN	+150[a] >400 n.c. n.c. n.c.	Intermediate[c]	Gouty, Brown, Rosenberger, & Cox, 2010
6-OHDA (mfb)	Rat	ppN/OFQ mRNA	SNc SNr STN striatum M1/M2 NAcc GP VPL/M	+150 +105 +45 –25 n.c. n.c. n.c. n.c.	Full[a]	Marti et al., 2010
6-OHDA (mfb), dyskinetic	Rat	N/OFQ binding	Striatum SNr/SNc STN M1/M2	+50 –50 n.c. n.c.	Full[a]	Marti et al., 2012
Paraquat + maneb (systemic)	Rat	ppN/OFQ mRNA	Striatum SN	n.c. +50	Low[b]	Bastias-Candia, Di Benedetto, D'Addario, Candeletti, & Romualdi, 2015
		NOP mRNA	Striatum SN	n.c. –40		
PD patients	—	ppN/OFQ mRNA	SN			Collins et al., 2015

Extent of lesion: full >90%, intermediate 50–80%, low <40%, as evaluated by [a] striatal TH immunohistochemistry,[b]TH levels in striatum or SN (Western blot), or [c]SNc dopaminergic neuron count. mfb, medial forebrain bundle; n.c., no change.
[a]Strain dependent.

N/OFQ-like immunoreactivity was monitored in dialysates obtained from the lesioned and unlesioned SNr of 6-OHDA hemilesioned rats (Marti et al., 2005). In line with expression data, N/OFQ-like immunoreactivity was threefold greater in the lesioned, compared with the unlesioned side, indicating that N/OFQ transmission was up-regulated in the SNr of the parkinsonian brain. Interestingly, in the same study, forcing animals to move on a rotating cylinder caused N/OFQ levels to rise, more markedly in the lesioned (+150%) than the unlesioned (+40%) SNr. This not only showed that endogenous N/OFQ release in SNr is under a tonically inhibitory dopaminergic control but also that N/OFQ release reflects ongoing neuronal activity and motor function. Consistent with this, functional impairment of dopaminergic transmission achieved with acute **haloperidol** administration resulted in elevation of N/OFQ levels (along with glutamate levels) in SNr (Marti et al., 2010). Although this elevation was mild (+50%), it suggested that N/OFQ release parallels striato-pallidal neuron activity. Indeed, the most parsimonious explanation is that the blockade of striatal **dopamine D_2 receptors** on striato-pallidal GABAergic neurons (the first neuron along the polysynaptic "indirect" pathway) by

haloperidol disinhibits subthalamic nucleus (STN) activity, causing an increase of N/OFQ release in SNr (Marti et al., 2010).

To confirm a possible role for N/OFQ in PD, CSF was collected in PD patients by cranial drainage during surgical implantation of electrodes for deep brain stimulation (Marti et al., 2010). In line with the findings in the dopamine-depleted rat SNr, a 3.5-fold elevation of N/OFQ levels in the CSF from PD, compared with non-PD patients was found (Marti et al., 2010). As human CSF is in equilibrium with parenchymal fluids, this study suggests that N/OFQ transmission is elevated in PD, although the source of N/OFQ in CSF remains to be identified.

4 | NOP RECEPTOR LIGANDS IN PD MODELS: SYMPTOMATIC EFFICACY

4.1 | The role of endogenous N/OFQ in motor control

Studies carried out in NOP receptor knockout mice ($NOP^{-/-}$; Marti et al., 2004; Viaro, Calcagno, Marti, Borrelli, & Morari, 2013) or in naïve rodents and non-human primates using ppN/OFQ-targeted anti-sense oligonucleotides (Candeletti & Ferri, 2000) and NOP receptor antagonists (Marti, Mela, Veronesi, et al., 2004; Marti, Viaro, Guerrini, Franchi, & Morari, 2009; Viaro et al., 2008; Viaro et al., 2013; Viaro, Marti, & Morari, 2010; Wallace, Bezard, Pioli, & Martin, 2019) show that endogenous N/OFQ tonically inhibits motor function. Modulation of the basal ganglia (BG) output is instrumental to movement control exerted by N/OFQ. Indeed, the motor effects elicited by i.c.v. injection of N/OFQ or the NOP antagonist UFP-101 in the rat were reproduced by intra-SNr injection of these molecules (Marti et al., 2009). Consistent with neuroanatomical connections of the BG (Albin, Young, & Penney, 1989; Alexander, Crutcher, & DeLong, 1990), i.c.v. or intra-SNr injection of N/OFQ or NOP receptor ligands evoked similar changes in the excitability of primary motor cortex and motor output, as evaluated by intracortical microstimulation (Marti et al., 2009). Contrary to the effect of N/OFQ, NOP receptor antagonists improved motor performance and increased primary motor cortex excitability, suggesting that nigral NOP receptor blockade inhibits the nigro-thalamic pathway and disinhibits the thalamo-cortical projections (Marti et al., 2009). Therefore, the increase of N/OFQ expression and release found in the SNr of parkinsonian animals might pose a further burden on motor function and contribute to PD symptoms. In line with this hypothesis, blockade of NOP receptors in the SNr appears to reduce motor deficits in rodent and non-human primate models of PD (vide infra).

4.2 | Models of functional parkinsonism: Genetic and pharmacological blockade of NOP receptors

Drugs that impair dopaminergic transmission, such as haloperidol or **reserpine**, are known to precipitate akinesia/bradykinesia without affecting the viability of nigrostriatal dopaminergic neurons (Duty & Jenner, 2011). Although these drugs do not phenocopy the neuropathology underlying PD, they can reproduce parkinsonian akinesia/bradykinesia and activate the same pathways underlying parkinsonian symptoms (Duty & Jenner, 2011). For instance, haloperidol (Mabrouk, Marti, & Morari, 2010; Marti et al., 2004) and reserpine (Volta, Mabrouk, Bido, Marti, & Morari, 2010) elevate glutamat levels in SNr, possibly reflecting STN disinhibition, that is, increase in the firing rate and/or burst activity of STN neurons (Degos et al., 2005; Robledo & Feger, 1991), culminating in thalamic inhibition and motor impairment (Albin et al., 1989; Alexander et al., 1990). Although phenomenologically more complex than in rodents, abnormal activity of STN neurons (along with synchronization in the β band) is also a feature of the human PD brain (Benazzouz et al., 2002; Bergman, Wichmann, Karmon, & DeLong, 1994; Hammond, Bergman, & Brown, 2007).

The contribution of endogenous N/OFQ to neuroleptic-induced PD-like akinesia/catalepsy was first revealed by the finding that unilateral intra-SNr microinjection of the NOP receptor antagonist UFP-101 rescued haloperidol-treated rats from akinesia, increased contralateral rotations, and simultaneously reversed the associated elevation of nigral glutamate release (Marti, Mela, Guerrini, et al., 2004). In line with these findings, $NOP^{-/-}$ mice were less prone than wild-type controls to develop motor impairment after administration of low doses (0.3–0.8 $mg \cdot kg^{-1}$) of haloperidol (Marti et al., 2005). Perhaps not too surprising, when higher haloperidol doses were used (1.5–3 $mg \cdot kg^{-1}$), the genotype difference disappeared. This might indicate that NOP receptor blockade provides therapeutic benefit only in conditions of early, mild parkinsonism. Nonetheless, consistent with data in the genetic model, UFP-101 (10 nmol, i.c.v.) or J-113397 (0.1–10 $mg \cdot kg^{-1}$, i.p.) improved motor impairment induced by haloperidol in naïve mice (Mabrouk et al., 2010). The effectiveness of NOP receptor antagonists was also demonstrated in reserpinized animals. Reserpine binds to **vesicular monoamine transporter type 2** and prevents the refilling of synaptic vesicles, thereby causing depletion of vesicular stores of dopamine and other monoamines which results in akinesia and depression (Schultz, 1982). Acute reserpine administration (0.1–3 $mg \cdot kg^{-1}$, i.p.) to mice causes a dose-dependent and long-lasting (>3 days) impairment of motor activity (Volta, Mabrouk, et al., 2010). Daily administration of J-113397 (1 $mg \cdot kg^{-1}$, i.p.) rescued motor disabilities induced by reserpine and significantly accelerated the recovery of motor function, progressively improving baseline values over time, in comparison with saline-treated animals. J-113397 also caused acute improvement of motor activity, which, however, developed tolerance within 2 days. Acute administration of SB-612111 (0.1–10 $mg \cdot kg^{-1}$) also dose-dependently improved motor activity in reserpinized mice, an effect partly replicated by NiK-21273 (Marti et al., 2013). Studies with reserpine in $NOP^{-/-}$ mice substantiated the beneficial effect of NOP receptor antagonists. In line with findings with haloperidol, genetic removal of the NOP receptor conferred resistance on mice to motor impairment induced by reserpine (Volta, Mabrouk, et al., 2010). Also, as observed with haloperidol, this resistance faded when higher doses of reserpine were injected.

4.3 | Models of neurotoxic parkinsonism: 6-OHDA hemi-lesioned rats

The proof of concept that NOP receptor antagonists possess symptomatic anti-parkinsonian properties was provided by Morari and collaborators in 6-OHDA hemi-lesioned rats (Marti et al., 2005), using UFP-101 and J-113397 in comparison with fixed doses of a positive control L-DOPA (1 mg·kg^{-1}). UFP-101 (0.1–30 nmol, intranigral) and J-113397 (0.1–3 mg·kg^{-1}, i.p.) reduced akinesia/bradykinesia and improved overall motor performance, reproducing the pattern of responses to L-DOPA. Other small-molecule NOP antagonists given i.p. also proved effective in this model: Trap-101 (Marti, Trapella, & Morari, 2008), GF-4 (Volta, Marti, et al., 2010), NiK-21273 (Marti et al., 2013), and the more potent and selective NOP antagonists C-24 (Volta, Viaro, Trapella, Marti, & Morari, 2011) and SB-612111 (Marti et al., 2013). All NOP antagonists improved motor function with similar efficacies but different potencies, C-24 being the most potent antagonist and Trap-101 the least potent. Notably, most compounds showed a bell-shaped profile, losing their positive effect or even causing overt motor inhibition at higher doses, as shown with J-113397, GF-4, and C-24 administered at 30 mg·kg^{-1}.

Prompted by the finding in reserpinized mice (Volta, Mabrouk, et al., 2010), tolerance to the anti-parkinsonian effect of SB-612111 was assessed in 6-OHDA hemilesioned rats (Marti et al., 2013). Two doses of SB-612111 (0.01 and 1 mg·kg^{-1}) were chronically administered for 16 days, and motor function assessed before (chronic effect) and after drug administration (acute effect). Essentially, there was no chronic effect of SB-612111 over motor function (i.e., no changes of baseline motor activity over time) and the acute effect of the higher dose remained unchanged during the study, indicating there was no development of tolerance. On the contrary, a chronic positive effect, that is, a late improvement of baseline motor function 12–16 days after the onset of administration, was observed with NiK-21273 (1.5 mg·kg^{-1}) but a rapid tolerance (within 4 days) developed to these acute effects.

From a clinical perspective, each new anti-parkinsonian drug should be tested for its ability to improve, or at least not worsen, the symptomatic effect of L-DOPA. Studies in rodents (and non-human primates, see below) revealed that NOP receptor antagonists additively or synergistically potentiate the effect of L-DOPA, depending on the compound and dose. In 6-OHDA hemi-lesioned rats, a fully effective dose of J-113397 (1 mg·kg^{-1}) additively improved the beneficial effects of a submaximal dose of L-DOPA (1 mg·kg^{-1}; Marti, Trapella, Viaro, & Morari, 2007). The reverse was also true, since a subthreshold (ineffective) dose of L-DOPA (0.1 mg·kg^{-1}, i.p.) potentiated the motor effects induced by maximally effective doses of Trap-101 (10 mg·kg^{-1}, i.p.; Marti et al., 2008). A marked synergistic interaction between subthreshold doses of SB-612111 (0.01 mg·kg^{-1}) and L-DOPA (0.1 mg·kg^{-1}) was also later demonstrated (Marti et al., 2013), an effect replicated by substituting SB-612111 with subthreshold doses of NiK-21273 (0.5 mg·kg^{-1}).

4.4 | Models of neurotoxic parkinsonism: MPTP-treated mice and non-human primates

MPTP administration in mice brings about motor changes that differ depending on MPTP doses, protocols of MPTP administration, and sensitivity of tests used to evaluate motor function. Due to these inconsistencies, MPTP-treated mice are not routinely used as a model for studying the symptomatic effect of novel putative anti-parkinsonian molecules, although motor deficits can be captured and quantified in these mice (Sedelis et al., 2000). A week after acute MPTP administration to mice (4 × 20 mg·kg^{-1}), an ~60% loss of striatal dopaminergic terminals occurs along with significant elevation of akinesia/bradykinesia and overall motor impairment (Viaro et al., 2008). J-113397 (0.01–0.03 mg·kg^{-1}) reversed these changes, an effect comparable to that of the positive control (L-DOPA 10 mg·kg^{-1}). In addition, a reversal of the J-113397 action was detected at 1 mg·kg^{-1}, consistent with the bell-shaped nature of the dose–response curve of this compound.

The effect of J-113397 was further confirmed in MPTP-treated, stably parkinsonian non-human primates (Viaro et al., 2008), the gold standard among preclinical models of PD. The motor performance of four macaques was evaluated via computerized time-reaching tasks (MAP test, i.e., the platform and straight rod tests) or by post hoc videotape analysis (UPDRS scale). Preliminary dosing in these animals indicated a positive effect of 0.01 mg·kg^{-1} J-113397 in the MAP test. This dose also caused symptomatic benefit in all four animals, improving various motor parameters such as hypokinesia, bradykinesia, tremor, balance, and rigidity. J-113397 was overall 50% less effective than L-DOPA (30 mg·kg^{-1}, i.m.), although it was as effective as L-DOPA on hypokinesia. As observed in rodent models, higher doses of J-113397 negatively affected behavior and, in particular, 1 mg·kg^{-1} J-113397 caused long episodes of freezing. A study in MPTP-treated marmosets (Visanji et al., 2008) revealed that 30 mg·kg^{-1} J-113397 (s.c.) potentiated the effect of a low sub-therapeutic dose (12.5 mg·kg^{-1}) dose of L-DOPA, however, at the cost of inducing dyskinesia. The authors raised the concern that the L-DOPA-sparing effect of NOP antagonists would be counterbalanced by the appearance of dyskinetic movements. Preliminary evidence that BTRX-246040 (0.1–1 mg·kg^{-1}, i.m.) improves motor symptoms in MPTP-treated macaques was recently reported, although this symptomatic effect was not consistently observed after subchronic exposure (Wallace et al., 2019). Nonetheless, these promising data formed the basis of the clinical evaluation of BTRX-246040 in Phase II trials for PD (NCT03608371, 2019).

4.5 | Models of α-synucleinopathy

The discovery that mutations in the SNCA gene coding for α-synuclein were associated with autosomal dominant PD (Polymeropoulos et al., 1997) and that Lewy bodies, a neuropathological feature of PD, are mainly composed of α-synuclein (Spillantini et al., 1997) paved the way for the research on the genetics of PD.

Thus, overexpression of native or mutated α-synuclein has been used to replicate the synucleinopathy observed in PD brains (Dehay, Vila, Bezard, Brundin, & Kordower, 2016; Koprich, Kalia, & Brotchie, 2017). SB-612111 has been investigated in a synucleinopathy model generated by the injection of a recombinant adeno-associated virus of serotype 2/9 (AAV2/9) carrying a PD-associated mutant of human α-synuclein (A53T) in the SNc of naïve rats (Bourdenx et al., 2015). Transgene expression was associated with nigrostriatal degeneration and progressive motor deficits, namely, reduction of stepping activity (Arcuri et al., 2016). SB-612111 (Arcuri et al., 2016) or BTRX-246040 (Wallace et al., 2019), administered daily for 8 weeks, starting a week after virus injection, prevented motor impairment, although these compounds had different neuroprotective effects (see below). Collectively, these data suggest NOP receptor antagonists exhibit beneficial effects on motor symptoms in preclinical PD models, and further exploration is warranted to establish the therapeutic potential of this approach for the treatment of PD.

5 | MECHANISMS OF SYMPTOMATIC ACTION OF NOP RECEPTOR ANTAGONISTS

The mechanism underlying the anti-parkinsonian action of NOP receptor antagonists appears to be their ability to reset the balance between excitatory and inhibitory inputs on GABAergic nigro-thalamic neurons, which ultimately lead to thalamic disinhibition and movement facilitation (Figure 1). According to the classical model of BG function (Albin et al., 1989; Alexander et al., 1990), the parkinsonian condition is associated with increased activity of the glutamatergic projections from the STN to the output nuclei of the BG, namely, SNr and the pars interna of the globus pallidus. Simultaneously, the loss of the positive influence of striatal D_1 receptor over glutamatergic inputs driving striatal medium-sized spiny neurons (MSNs) projecting to the SNr/globus pallidus pars interna (the so-called direct pathway) makes them hypoactive. These network changes result in a net increase of the excitatory drive over the tonically active nigrofugal GABAergic neurons, leading to further inhibition of thalamo-cortical projections.

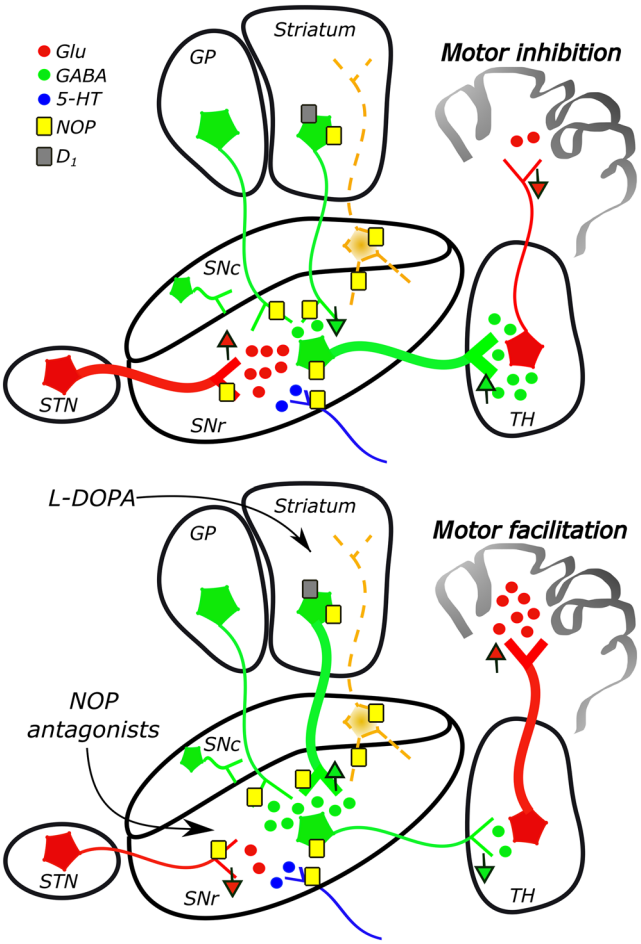


FIGURE 1 Neurocircuitry and neurotransmitter changes in PD (upper panel) and during therapeutic intervention with NOP antagonists (lower panel). The degeneration of dopaminergic neurons located in substantia nigra compacta (SNc) in PD results in increased activity of glutamatergic neurons of subthalamic nucleus (STN) and decreased activity of D_1 receptor-expressing striatonigral GABAergic neurons. The consequent overactivation of GABAergic neurons projecting from substantia nigra reticulata (SNr) to thalamus (TH) and inhibition of glutamatergic thalamo-cortical projections results in parkinsonian hypokinesia (upper panel). NOP receptor antagonists, by acting on NOP receptors located both presynaptically and postsynaptically as shown (yellow squares), reset the balance between excitatory and inhibitory inputs impinging on nigro-thalamic neurons, leading to motor improvement (lower panel). As discussed, NOP receptor blockade in substantia nigra reticulata (SNr) reduces glutamate and elevates GABA levels in SNr, resulting in reduction of GABA levels in the ventromedial thalamus. However, high doses of NOP antagonist produce opposing effects. Thickness and arrows indicate increased or decreased pathway activity and neurotransmitter release

5.1 | NOP receptor antagonists and glutamate release

Microdialysis studies in rodents consistently demonstrated that NOP receptor antagonists reduced glutamate release in the SNr of parkinsonian animals. As discussed earlier in this review, haloperidol-induced akinesia/catalepsy is associated with elevation of nigral glutamate release (Mabrouk et al., 2010; Marti, Mela, Guerrini, et al., 2004) most likely due to disinhibition of the subthalamo-nigral pathway (Degos et al., 2005). UFP-101 (10 nmol, i.c.v.) and J-113397 (1 mg·kg^{-1}, i.p.) reversed haloperidol-induced nigral glutamate release (and the accompanying akinesia) in the rat (Marti et al., 2005; Marti, Mela, Guerrini, et al., 2004) and in the mouse (Mabrouk et al., 2010). Consistent with this, genetic deletion of the NOP receptor also attenuated haloperidol-induced elevation of nigral glutamate release and the associated catalepsy (Mabrouk et al., 2010). Confirming the close relationship between catalepsy, endogenous N/OFQ and glutamate in SNr, a microdialysis study in both NOP$^{-/-}$ and NOP$^{+/+}$ mice treated with reserpine, found an increase in nigral glutamate release and

immobility time, although both effects were milder in $NOP^{-/-}$ mice (Volta, Mabrouk, et al., 2010).

Microdialysis studies in 6-OHDA rats substantially confirmed the ability of NOP receptor antagonists to reduce nigral glutamate. In fact, in a microdialysis study where probes were simultaneously implanted in the lesioned and unlesioned SNr, UFP-101 (1–10 μM through the probe) or J-113397 (0.1–3 $mg{\cdot}kg^{-1}$, i.p.) reduced glutamate levels by ~20–30% in both hemispheres, although more potently in the lesioned one. Similar reductions of basal glutamate release were observed after systemic administration of Trap-101 (10 $mg{\cdot}kg^{-1}$; Marti et al., 2008) and GF-4 (1 $mg{\cdot}kg^{-1}$; Volta, Marti, et al., 2010), or local perfusion of Trap-101 (10 μM; Marti et al., 2008) and C-24 (0.03 μM; Volta et al., 2011) through a microdialysis probe implanted in the SNr. Concurrent behavioural monitoring in animals subject to microdialysis confirmed that these pharmacological procedures led to significant attenuation of akinesia. Overall, these data suggest that dopamine loss amplifies a tonic, conceivably indirect, excitatory action of endogenous N/OFQ over nigral glutamate terminals (Marti, Guerrini, Beani, Bianchi, & Morari, 2002). As an increase of excitatory input over nigro-thalamic GABA neurons causes thalamic inhibition and impairment of motor initiation, it is plausible that NOP receptor antagonists reduce akinesia by blocking this action.

5.2 | NOP receptor antagonists and GABA release

The resetting of glutamate inputs in the SNr is perhaps not the only mechanism through which NOP receptor antagonists ameliorate parkinsonian motor symptoms. In fact, in vivo microdialysis also showed that NOP receptor antagonists increase GABA levels in the SNr, which might thus reinstate the physiological inhibitory control operated by the striatonigral direct pathway over nigro-thalamic neurons (Albin et al., 1989; Alexander et al., 1990). The first evidence of this was obtained in 6-OHDA hemi-lesioned rats where simultaneous administration of L-DOPA and J-113397 (1 $mg{\cdot}kg^{-1}$) additively improved motor function and elevated nigral GABA levels (Marti et al., 2007), an effect that was prevented by the voltage-dependent sodium channel blocker **tetrodotoxin** (TTX), indicating the neuronal source of GABA levels measured by microdialysis. This GABA-facilitating effect was later replicated by systemic administration of Trap-101 (10 $mg{\cdot}kg^{-1}$; Marti et al., 2008) and GF-4 (1 $mg{\cdot}kg^{-1}$; Volta, Marti, et al., 2010) as well as by reverse dialysis of Trap-101 (10 μM; Marti et al., 2008) and C-24 (3 μM; Volta et al., 2011) in the SNr, confirming that NOP receptors located in SNr tonically inhibit GABA release in this area.

5.3 | NOP receptor antagonists and nigro-thalamic GABAergic transmission

The proof that the changes of glutamate and GABA levels induced by NOP antagonists in the SNr affect the activity of nigro-thalamic GABAergic neurons and consequently motor function was provided by dual probe microdialysis studies (Marti et al., 2007; Marti et al., 2008; Volta et al., 2011; Volta, Marti, et al., 2010). In these studies, one probe was implanted in the lesioned SNr and another in the ipsilateral ventro-medial thalamus (VMTh), a target of nigral projections. The finding that intranigral perfusion with TTX reduced (~30%) GABA release in VMTh and simultaneously improved akinesia (Marti et al., 2007) was consistent with the view that inhibition of nigro-thalamic GABAergic neurons disinhibits thalamo-cortical projections (Albin et al., 1989; Alexander et al., 1990). Similar effects were induced by the combination of L-DOPA and J-113397 that, in addition to elevating GABA and reducing glutamate in SNr (see above), also reduced GABA in VMTh (Marti et al., 2007). These effects were occluded by TTX, suggesting these neurochemical changes reflected ongoing neuronal activity and the involvement of nigro-thalamic neurons. The involvement of the nigro-thalamic pathway in the motor-promoting action of NOP antagonists was further confirmed by reverse dialysis of the **$GABA_A$ receptor** antagonist **bicuculline** in SNr (Marti et al., 2007), based on the reasoning that if an elevation of GABA in SNr was responsible for the inhibition of nigro-thalamic neurons, blockade of nigral $GABA_A$ receptors expressed by nigro-thalamic neurons would prevent this effect. Consistent with this hypothesis, bicuculline did not block the rise of nigral GABA induced by L-DOPA plus J-113397 but prevented its inhibitory effect over thalamic GABA levels (Marti et al., 2007). Again, the neurochemical effects of bicuculline were accompanied by significant behavioural changes, that is, blockade of the anti-akinetic effect, confirming that $GABA_A$ receptors on nigro-thalamic neurons need to be activated to enable the anti-akinetic effect of NOP receptor antagonists (Marti et al., 2007). As J-113397 was administered systemically in this study, later microdialysis experiments where NOP antagonists were directly perfused into SNr were carried out to convincingly demonstrate that blockade of nigral NOP receptors promotes movement by over-inhibiting the nigro-thalamic GABAergic projection. Thus, reverse dialysis of Trap-101 (Marti et al., 2008) and C-24 (Volta et al., 2011) in SNr not only reduced nigral glutamate and elevated nigral GABA (see above) but also reduced thalamic GABA and attenuated akinesia. Further, Trap-101, combined with L-DOPA, produced a larger reduction of thalamic GABA (Marti et al., 2008).

Reverse dialysis of C-24 in SNr also provided valuable information on the mechanisms underlying the *motor inhibiting* effect caused by high doses of NOP receptor antagonists (Volta et al., 2011). Perfusion with a high concentration (3 μM) of C-24 increased akinesia, simultaneously reducing GABA release in SNr and elevating GABA release in VMTh (a tendency for an elevation of glutamate release in SNr was also evident). This represents a neurochemical pattern opposite to that evoked by the 100-fold lower, 0.03-μM anti-akinetic concentration of C-24, indicating that motor inhibition induced by high doses of NOP receptor antagonist is associated with nigro-thalamic pathway activation (and thalamic inhibition).

6 | NOP RECEPTOR LIGANDS IN PD MODELS: NEUROPROTECTIVE EFFICACY

Cox and collaborators provided the first evidence that endogenous N/OFQ contributes to parkinsonian degeneration (Marti et al., 2005). In fact, ppN/OFQ$^{-/-}$ mice were reported to be more resistant than ppN/OFQ$^{+/+}$ mice, to the neurotoxic action of acute MPTP, showing a greater number of dopaminergic neurons and striatal dopaminergic terminals spared a week after acute MPTP administration (Marti et al., 2005). Interestingly, N/OFQ does not affect methamphetamine-induced neurotoxicity, which is mainly targeted to striatal terminals, suggesting N/OFQ could facilitate MPTP-induced toxicity acting at the nigral level (Brown, Gouty, Iyer, Rosenberger, & Cox, 2006). As ppN/OFQ codes for two other biologically active neuropeptides besides N/OFQ, that is, N/OFQ II and nocistatin, it was mandatory to investigate the toxicity of endogenous N/OFQ in NOP$^{-/-}$ mice (Arcuri et al., 2016). Indeed, NOP$^{-/-}$ mice responded to acute MPTP exactly as ppN/OFQ$^{-/-}$ mice, showing greater resistance to the toxin than NOP$^{+/+}$ mice (Arcuri et al., 2016). The greater resistance to MPTP was also accompanied by better motor performances in the bar and drag tests (Arcuri et al., 2016). The idea that endogenous N/OFQ could play a neurotoxic role in PD was further corroborated using a clinically driven study design in more progressive PD models, which allow a window for therapeutic intervention. In these experiments, SB-612111 was used, and its administration was delayed with respect to the neurotoxic insult, as it occurs in the clinic, where the patient comes to the attention of the neurologist when motor symptoms appear, that is, far later than when the disease has started its course. Using such a protocol, SB-612111 (10 mg·kg^{-1}, given twice daily for 10 days, starting at the fourth day after the administration of MPTP) was capable of preventing the nigrostriatal degeneration induced by subacute MPTP administration (25 mg·kg^{-1}, i.p., once daily for 7 days; Arcuri et al., 2016). Moreover, SB-612111 (1 mg·kg^{-1}, twice daily for 8 weeks, commencing a week after AAV2/9 h α-synuclein injection) also attenuated the nigrostriatal neurodegeneration induced by α-synuclein overexpression. The percentage of dopaminergic cells spared was significantly greater in SB-612111-treated (50%) than in saline-treated (25%) rats. Considering that about 50% of nigral dopaminergic cells die a week after injection of AAV2/9 h α-synuclein injection (Bourdenx et al., 2015), the neuroprotective effect of SB-612111 on dopaminergic cells is indeed a remarkable result. Independent confirmation of neuroprotective effect of SB-612111 in the very same α-synuclein overexpression rat model was recently obtained in a head-to-head comparison of BTRX-246040 and SB-612111. BTRX-246040, SB-612111, and vehicle were administered s.c. twice daily for 7 weeks, starting on Day 7 after AAV administration (Wallace et al., 2019). While both NOP receptor antagonists had a positive effect on locomotor activity (see above) and interfered with α-synuclein levels suggesting disease-modifying activity, only SB-612111 demonstrated neuroprotective properties whereas BTRX-246040 did not match this effect but displayed an intermediate profile between the vehicle and the SB-612111 group. Further investigation is warranted to confirm the neuroprotective effect of NOP antagonists in animal models and the therapeutic potential of this approach for treatment of PD.

These in vivo data strongly suggest that endogenous N/OFQ is harmful for dopaminergic neurons. This was confirmed in vitro (Collins et al., 2015). N/OFQ and the NOP agonist UFP-112 potentiated the toxic action of 6-OHDA on SH-SY5Y cell viability (Collins et al., 2015). Remarkably, N/OFQ inhibited survival and complexity (neurite length and branching) in primary cultures of dopaminergic neurons, potentiating the effects of the parkinsonian toxins MPP$^+$ and 6-OHDA (Collins et al., 2015). N/OFQ effects were observed at relatively low concentrations (10–500 nM) and were specifically reversed by SB-612111, again indicating they were mediated by NOP receptors.

Studies are ongoing to identify the mechanisms underlying the neurotoxic pathways activated by N/OFQ. As exogenous N/OFQ elevates glutamate release in the rodent SNr whereas NOP receptor antagonists reduce it (see above), we first hypothesized that endogenous N/OFQ can cause dopaminergic neuron degeneration through glutamate-mediated excitotoxicity (Brown et al., 2006; Marti et al., 2005). Indeed, changes in mitochondrial potential due to inhibition of complex I by MPP$^+$ (the active metabolite of MPTP) lead to oxidative stress and glutamate-mediated excitotoxicity, which contribute to degeneration of dopaminergic neurons (Meredith & Rademacher, 2011; Serra et al., 2002). Alternative mechanisms might involve a modulation of the immune response as neuroinflammation plays an important role in neurodegeneration (Nolan, Sullivan, & Toulouse, 2013; Poewe et al., 2017) and there is evidence that N/OFQ causes microglial activation (Laudenbach et al., 2001). However, both pro- and anti-inflammatory effects of N/OFQ have been described (Mallimo & Kusnecov, 2013). The NOP receptor seems to bidirectionally modulate the expression and release of cytokines. In particular, N/OFQ has been shown to inhibit the production of pro-inflammatory cytokines such as **IL-6**, **IL-1β**, and **TNFα** in different tissues and cell types, including glial cells. On the other hand, prolonged activation of the NOP receptor causes a dramatic activation of NF-kB, a key modulatory transcription factor of the pro-inflammatory response (Toll et al., 2016). How the NOP receptor exerts its action on microglia is not clear. As cytokine activation and NOP receptor signalling share a common transduction pathway, that is, the **MAPK** pathway, we can speculate crosstalk between NOP and cytokine signalling in the modulation of the inflammatory response. Preliminary data in support of this hypothesis come from the study of O'Keeffe and colleagues (Collins et al., 2015), showing that N/OFQ inhibits the survival and growth of primary cultures of dopaminergic neurons through the **p38**-MAPK cascade. The p38-MAPK signalling is implicated in different neurodegenerative diseases through its regulatory action on apoptosis and inflammation (Cuenda & Rousseau, 2007; Zarubin & Han, 2005) and increased phospho-p38 levels have been shown in the SNc dopaminergic neurons of PD patients (Karunakaran et al., 2008).

7 | NOP RECEPTOR AGONISTS IN L-DOPA-INDUCED DYSKINESIA

A role for enhanced peptidergic neurotransmission, either opioidergic or not, has classically been proposed for the generation of L-DOPA-induced dyskinesia (LID), a debilitating complication of L-DOPA therapy for PD (Bastide et al., 2015), mostly on the basis of in situ hybridization studies showing that striatal peptidergic precursor expression consistently correlates with LID severity (Aubert et al., 2007; Cenci, Lee, & Bjorklund, 1998; Henry, Duty, Fox, Crossman, & Brotchie, 2003; Tel et al., 2002). The N/OFQ–NOP receptor system had however received little attention until recent years, with the increased understanding of its complex role in BG pathophysiology. Just as up-regulation of the nigral N/OFQ–NOP receptor system is targeted by NOP receptor antagonists resulting in a symptomatic anti-parkinsonian effect (Marti et al., 2005), down-regulation of the striatal N/OFQ–NOP receptor system (Marti et al., 2012) can be overcome by NOP receptor agonists, resulting in a symptomatic effect on LID, as shown in both rodent and non-human primate models of PD (Arcuri et al., 2018; Marti et al., 2012).

Intracerebral N/OFQ itself or systemic administration of the NOP receptor agonist Ro 65-6570 dampened LID severity in rats, with an equal effect on axial, limb, and orolingual abnormal involuntary movements (Marti et al., 2012), the rodent correlates of LID in PD models. Worth noting is the observation that the anti-dyskinetic effect of NOP agonists did not interfere with the L-DOPA-mediated anti-parkinsonian effect and occurred at much lower doses than those reported to cause hypolocomotion in normal animals (Devine et al., 1996; Jenck et al., 1997; Marti et al., 2009; Marti, Mela, Guerrini, et al., 2004). As NOP receptor agonists substantially reversed LID severity in the macaque model of PD, with an equal impact on choreic-like and dystonic-like dyskinesia components, without impairing the anti-parkinsonian action of L-DOPA (Marti et al., 2012), the use of NOP agonists for managing LID severity is strongly supported by experimental evidence. However, this view is somewhat questioned by the finding that the NOP antagonist BTRX-246040 also showed a capacity to alleviate LID, along with parkinsonian motor deficits in macaques, although subchronic administration failed to replicate the acute findings (Wallace et al., 2019). A comprehensive report of this study is awaited to ascertain whether both NOP receptor agonists (Ro 65-6570) and antagonists (BTRX-246040) should relieve LID, although a similar picture has been described for nicotinic receptor agonists and antagonists which reduce cholinergic signals in striatum through receptor desensitization or blockade respectively (Bordia, Campos, Huang, & Quik, 2008; Bordia & Perez, 2019). Nonetheless, further studies with more selective NOP agonists confirm the view that NOP receptor agonists have anti-dyskinetic properties in rodents. The NOP selective agonist AT-403 was able to attenuate LID in 6-OHDA hemi-lesioned rats (Arcuri et al., 2018). In this study, however, the anti-dyskinetic effect of AT-403 partly superimposed with a sedative/hypo-locomotive effect, which markedly reduced the therapeutic window. However, despite this narrow effective window, the anti-dyskinetic dose did not worsen parkinsonian-like disabilities but improved akinesia in L-DOPA-naïve 6-OHDA rats (Arcuri et al., 2018). One can still investigate the possibility of combining NOP agonists with the current anti-dyskinetic benchmark treatment, **amantadine** (Stanley, Pioli, Kozak, Popiolek, & Bezard, 2018) as well as study the possibility that NOP agonists might delay or prevent the development of LID. In this respect, only AT-403 was tested chronically, in the classical 21-day LID induction protocol (Arcuri et al., 2018). However, AT-403 prevented LID only at first testing, without affecting the overall dyskinesia profile. Whether this short-lived effect is due to the development of NOP receptor desensitization remains to be determined.

7.1 | Mechanisms of the anti-dyskinetic action of NOP receptor agonists

In contrast to NOP receptor antagonists, N/OFQ attenuated dyskinesia more potently when injected in the striatum than in the SNr (Marti et al., 2012) highlighting the key role of up-regulated NOP receptors expressed by MSNs (Marti et al., 2012). in vivo microdialysis showed that N/OFQ attenuated LID expression by reducing striatal GABAergic MSNs activity (Marti et al., 2012). Those MSNs project to SNr, and N/OFQ significantly decreased the typical rise in SNr GABA release associated with LID expression (Bastide et al., 2015; Bido, Marti, & Morari, 2011; Mela et al., 2007; Mela, Marti, Bido, Cenci, & Morari, 2012), similar to the neurochemical profile of amantadine (Bido et al., 2011). Such a rise in GABA levels in LID is the consequence of the overactivation of striatal D_1 receptors (Bastide et al., 2015; Mela et al., 2012), as demonstrated by the finding that intrastriatal perfusion with a selective D_1 receptor antagonist prevented both the surge in nigral GABA and the accompanying dyskinetic behaviours (Mela et al., 2012). Interestingly, N/OFQ also prevented the reduction of GABA release in the SNr projection-receiving thalamus, associated with LID (Marti et al., 2012). The now classic up-regulation of striatal D_1 receptor signalling in LID (Aubert et al., 2005) is associated with several biochemical markers and, notably, with increased activity of the Ras/MEK/**ERK** pathway (Bastide et al., 2015; Feyder, Bonito-Oliva, & Fisone, 2011; Valjent et al., 2005; Figure 2). Consistent with an inhibitory action of N/OFQ on striatal D_1 receptor signalling (Olianas, Dedoni, Boi, & Onali, 2008), application of N/OFQ or the NOP agonist AT-403 to striatal slices of naïve animals prevented the increase in ERK phosphorylation induced by a D_1 receptor agonist (Arcuri et al., 2018; Marti et al., 2012). Such dampening of ERK signalling was confirmed in vivo using AT-403 in the rat model of PD and LID (Arcuri et al., 2018).

LID pathophysiology is also associated with the inability of cortico-striatal synapses to de-potentiate, leading to aberrant electrophysiological responses of D_1 receptor-expressing MSNs (Baufreton et al., 2018; Picconi et al., 2003). Electrophysiological investigations in brain slices of dyskinetic rats showed that N/OFQ fully restored depotentiation in slices treated with a D_1 receptor agonist (Marti

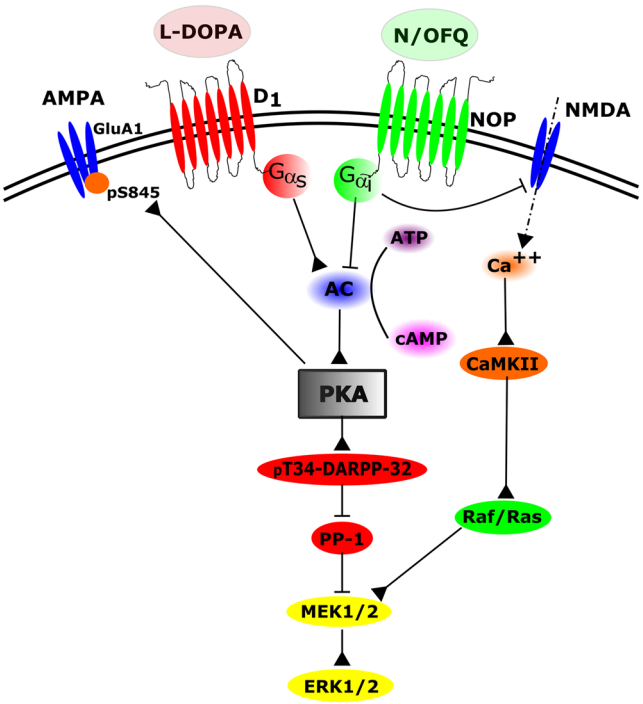


FIGURE 2 Mechanisms underlying NOP receptor-mediated inhibition of L-DOPA-induced dyskinesia (LID). LID is associated with sensitized D_1 receptor transmission at striatonigral GABAergic medium-sized spiny neurons (MSNs). D_1 receptor stimulation activates AC, which increases cAMP levels and activates PKA. PKA directly phosphorylates the AMPA receptor subunit GluA1 and DARPP-32 at Serine845 (S845) and Threonine34 (T34) residues respectively. pT34-DARPP-32 inhibits protein phosphatase-1 (PP-1), activating MAPK/ERK kinase (MEK) that, in turn, phosphorylates ERK. Crosstalk between D_1 and NMDA receptors also leads to activation of ERK through the Ca^{2+}/CaMKII/Raf-Ras/MEK signalling cascade. NOP receptor agonists might prevent D_1 receptor-mediated ERK phosphorylation through direct inhibition of AC and/or via inhibition of Ca^{2+} inward flux through the NMDA receptor

et al., 2012). Altogether, these data speak in favour of the use of NOP agonists to counter LID by restoring the inhibitory control over D_1 receptor signalling. Studies with more potent and selective NOP agonists are needed to confirm these observations.

8 | NOP RECEPTOR LIGANDS UNDER A CLINICAL PERSPECTIVE

PD is a multisystemic neurodegenerative disorder characterized by the loss of central and peripheral dopaminergic and nondopaminergic neurons, which causes the emergence of motor and non-motor symptoms (NMS; Poewe et al., 2017). Cardinal motor symptoms of PD, such as hypokinesia, bradykinesia, tremor, and rigidity, are due to reduction of dopaminergic levels in striatum and respond well to dopaminergic replacement therapy (Dragasevic-Miskovic, Petrovic, Stankovic, & Kostic, 2019). NMS instead generate from the loss of cholinergic, noradrenergic, and serotonergic neurons and are managed, although often unsatisfactorily, with non-dopaminergic drugs (Seppi et al., 2019). NMS affect neuropsychiatric behaviour, cognition, autonomic function, sensory system, and sleep and can precede the appearance of motor symptoms although they are also common in late-stage disease, resulting in significant disability (Barone et al., 2009). PD is currently managed with symptomatic medications that supplement lost dopaminergic function; however, these dopaminergic drugs are not effective against NMS or the underlying neurodegenerative process. In addition, L-DOPA, the cornerstone of PD pharmacotherapy, causes the appearance of wearing off and dyskinesias in more than 50% of patients after 5 years (Bastide et al., 2015; Bjornestad, Tysnes, Larsen, & Alves, 2016). Clinicians thus have to balance between effective control over motor symptoms and complications.

The lack of effective disease-modifying therapy or therapeutic agents targeted against PD-associated NMS or side effects of existing dopaminergic therapies makes the identification of novel targets and the development of new approaches for PD an urgent and critical need. In this respect, NOP receptor antagonists might represent a novel tool to counteract the neurodegeneration associated with PD. The fact that they are effective not only against dopaminergic neurotoxins (6-OHDA and MPTP) but also against neurotoxic triggers etiologically closer to idiopathic PD, such as α-synuclein overexpression, would indicate they interfere with the neurodegeneration process in dopaminergic and non-dopaminergic neurons, and perhaps glial cells. Here, it appears mandatory to clearly identify the mechanism(s) associated with their neuroprotective effect. Their ability to modulate the p38-MAPK and neuroinflammatory pathways, disclosed by preclinical studies (Collins et al., 2015; Mallimo & Kusnecov, 2013), would make NOP receptor antagonists a suitable tool not only for PD but also other neurodegenerative disorders. In addition to disease-modifying actions, NOP receptor antagonists might provide a broad spectrum of beneficial effects over both motor and non-motor functions (Figure 3), as the N/OFQ–NOP receptor system modulates the release of various neurotransmitters (Schlicker & Morari, 2000) and central and peripheral functions (Lambert, 2008; Toll et al., 2016). In particular, as discussed earlier, N/OFQ is capable of inhibiting noradrenaline 5-HT, dopamine and ACh release via presynaptic NOP receptors (Marti, Mela, Veronesi, et al., 2004; Mela et al., 2004; Schlicker & Morari, 2000), and NOP receptor antagonists reverse this effect, causing an increase in the release of such neurotransmitters. These non-dopaminergic actions might positively affect several NMS in PD, although the generalized, non-specific blockade of NOP receptors might generate off-target effects. For instance, it is well known that NOP receptor antagonists proved effective in rodent models of depression (Gavioli & Calo, 2013), and preliminary data that the NOP antagonist BTRX-246040 provides some anti-depressant effects in humans with major depressive disorder have been presented (Post et al., 2016). As depression is observed in a significant proportion of PD patients (Pagano & Politis, 2018; Zhuo et al., 2017), NOP receptor antagonists might improve depressive mood along with motor symptoms in PD. On the other hand, preliminary studies in major

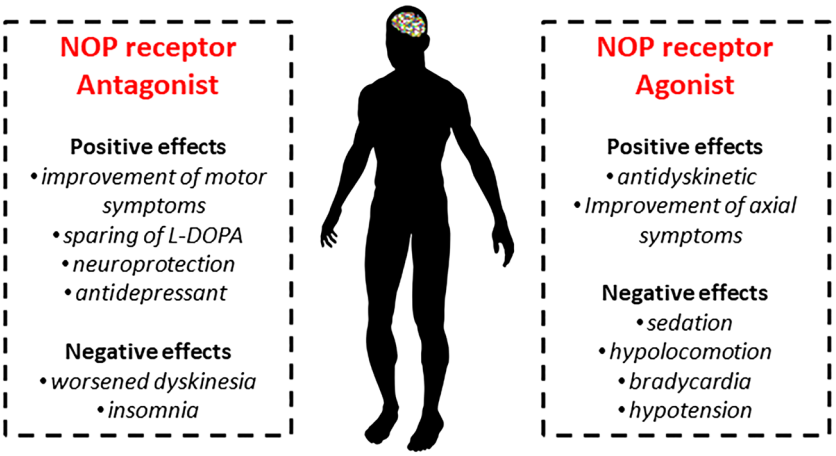


FIGURE 3 Putative effects of NOP receptor ligands in Parkinson's disease

depressive disorder patients also showed that BTRX-246040 caused insomnia, which suggests that NOP antagonism might worsen sleep disturbances typically seen in PD patients. At the moment, it appears difficult to anticipate the clinical profile of a NOP receptor antagonist since, apart from BTRX-246040, no other NOP receptor antagonist has been tested in humans. There is the question of how an NOP receptor antagonist could therapeutically target brain regions or functions relevant for the disease without interfering with NOP-mediated physiological functions and causing side effects. We can speculate that brain functions strongly relying on endogenous N/OFQ transmission or pathologically altered due to disease-associated up-regulation of endogenous N/OFQ transmission (typically, motor function involving nigral NOP receptors in rodents) would be the first targets of a NOP receptor antagonist. Thus, target selectivity will be determined by the levels of endogenous N/OFQ and occupancy of the NOP receptor in specific brain areas or synapses and can be achieved by a careful dose titration. Dose titration might be necessary to prevent possible pro-dyskinetic effect of a NOP receptor antagonist because, based on preclinical data in rodents (Marti et al., 2012) and marmosets (Visanji et al., 2008), NOP receptor antagonists are expected to potentiate the effect of L-DOPA, which, on the one hand, would result in a significant L-DOPA sparing effect and, on the other, might worsen LID. From a clinical perspective, an NOP receptor partial agonist (Khroyan et al., 2007; Ross et al., 2015) might be advantageous over a pure NOP receptor antagonist. In fact, such a compound might therapeutically block NOP receptors in areas where N/OFQ is pathologically up-regulated and provide a certain level of NOP receptor activity in those areas where endogenous N/OFQ mediates physiological actions or even rescue NOP receptor activity in those areas where N/OFQ transmission is pathologically impaired. Other ways to discriminate among different populations of NOP receptors and achieve target selectivity are at the moment very speculative. Subtle differences in in vivo pharmacology among NOP receptor antagonists exist, as shown by the fading of the motor-stimulant effects of NiK-21273 but not SB-612111 under subchronic administration, or the different ranges between motor stimulating and motor inhibiting doses among NOP receptor antagonists, or even the different neuroprotective effects of SB-612111 and BTRX-246040. However, the experimental evidence accumulated thus far points to the existence of only one class of NOP receptors, so these differences might be ascribed to yet unidentified pharmacodynamics or pharmacokinetic properties of NOP receptor antagonists. To add further complexity, biased NOP ligands, that is, ligands capable of differentially targeting the NOP-associated G_i rather than the β-arrestin 2 pathways, have been synthesized (Asth et al., 2016; Chang et al., 2015; Malfacini et al., 2015). Indeed, this strategy holds great promise for widening the therapeutic window of μ-receptor agonists (Azzam, McDonald, & Lambert, 2019). Thus, biased ligands of NOP receptors can help elucidate the biological effects mediated by NOP receptors and perhaps discriminate between different populations of NOP receptors. This action might be crucial for NOP receptor agonists in order to selectively target striatal NOP receptors and exert anti-dyskinetic effects. LID is the major motor complication in patients with PD (Espay et al., 2018), but it is difficult to categorize dyskinesias as peak dose or diphasic, given the phenomenological overlap. The ability of NOP receptor agonists to inhibit D_1 receptor signalling in PD models provides a solid rationale for their use in tackling peak-dose dyskinesia (Figure 3). Moreover, studies in dyskinetic macaques treated with NOP agonist Ro 65-6570 revealed a significant improvement of axial symptoms along with dyskinesia (Marti et al., 2012), which may clinically translate to gait improvement. Based on preclinical data, however, sedation and hypolocomotion might be common side effects. Bradycardia and hypotension (Burmeister, Ansonoff, Pintar, & Kapusta, 2008) may be other possible side effects that might worsen an already compromised cardiovascular function in PD patients (Palma & Kaufmann, 2018).

9 | CONCLUSIONS

Major unmet needs in the field of PD are the lack of a disease-modifying therapy, the poor pharmacological control exerted over NMS, and the lack of a drug preventing the development of motor complications associated with L-DOPA therapy, such as dyskinesia. Preclinical data strongly suggest that the N/OFQ–NOP receptor system would be a novel target for PD therapy. Selective and potent NOP receptor antagonists have been developed and characterized,

among which is the first orally active NOP receptor antagonist BTRX-246040. Such compounds might provide both symptomatic and neuroprotective/neurorescue benefits, also acting as L-DOPA sparing agents. Although the risk of worsening LID in advanced PD patients, or accelerating dyskinesia development in de novo PD patients, should be fully evaluated; this risk might be mitigated by careful titration of the dose of NOP receptor antagonist. Interestingly, the therapeutic benefit afforded by a NOP antagonist might extend to cover NMS, in particular, depression in PD patients.

The picture appears more complex for NOP receptor agonists as the compounds available so far, do not possess optimal pharmacological properties, particularly selectivity for NOP over μ receptors. Nonetheless, preclinical data point to a beneficial effect of NOP receptor agonists in attenuating peak-dose dyskinesia following acute administration, although the narrow therapeutic window of the most potent compounds might be a concern. Also, whether these compounds have the ability to prevent priming to L-DOPA over chronic treatment remains to be demonstrated.

The Phase II clinical trial testing the symptomatic efficacy of the NOP antagonist BTRX-246040 in PD will be pivotal in validating that NOP receptor antagonists might really represent a new hope for PD patients (Arcuri, Mercatelli, & Morari, 2017).

9.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20: (Alexander, Christopoulos et al., 2019; Alexander, Fabbro et al., 2019; Alexander, Kelly et al., 2019; Alexander, Mathie et al., 2019).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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