Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

DOPAMINE D2L RECEPTOR DENSITY INFLUENCES THE RECRUITMENT OF B-ARRESTIN2 AND GI1 INDUCED BY ANTIPARKINSONIAN DRUGS

Mattia Ferraiolo [a], Hicham Atik [a], Romane Ponthot [a], Beryl Koener [a], Julien Hanson [b,c], Emmanuel Hermans [a]

[a] *Neuropharmacology Laboratory - Institute of Neurosciences – UCLouvain, Brussels, Belgium*
[b] *Laboratory of Molecular Pharmacology – GIGA-Molecular Biology of Disease – ULiège, Liège, Belgium*
[c] *Laboratory of Medicinal Chemistry – CIRM – ULiège, Liège, Belgium*

KEYWORDS : Dopamine ; D_{2L} ; G protein ; β-arrestin ; Signaling bias ; Functional selectivity ; Antiparkinsonian ; Receptor density ; Parkinson's disease ; Luciferase ; G protein-coupled receptor

ABSTRACT

Introduction

Brain imaging studies have highlighted that the density of dopamine D_2 receptors markedly fluctuates across the stages of Parkinson's disease and in response to pharmacological treatment. Moreover, receptor density constitutes a molecular determinant for the signaling profile of D_2 receptor ligands. We therefore hypothesized that variations in receptor expression could influence D_2 receptor response to antiparkinsonian drugs, most notably with respect to the recruitment bias between G_{i1} and β-arrestin2.

Methods

The recruitment bias of dopamine, pramipexole, ropinirole, and rotigotine was examined using a nanoluciferase-based biosensor for probing the interactions of the D_{2L} receptor with either G_{i1} or β-arrestin2. The characterization of the functional selectivity of these D_2 receptor agonists was performed at two distinct D_{2L} receptor densities by taking advantage of a cell model carrying an inducible system that enables the overexpression of the D_{2L} receptor when exposed to doxycycline.

Results

A high receptor density oriented the balanced signaling profile of dopamine towards a preferential recruitment of G_{i1}. It also moderated the marked G_{i1} and β-arrestin2 biases of pramipexole and rotigotine, respectively. At variance, the G_{i1} bias of ropinirole appeared as not being influenced by D_{2L} receptor density.

Conclusions

Taken together, these observations highlight receptor density as a key driver of the signaling transducer recruitment triggered by antiparkinsonian agents. Moreover, given the putative beneficial properties of β-arrestin2 in promoting locomotion, this study provides molecular insights

Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

that position the arrestin-biased ligand rotigotine as a putatively more beneficial D_2 receptor agonist for the treatment of early and late Parkinson's disease.

1. Introduction

The commonly adopted first-line pharmacological treatments for Parkinson's Disease (PD) aim at treating motor deficits through the administration of the dopamine precursor levodopa or non-ergot D_2 receptor agonists such as pramipexole, ropinirole, or rotigotine (Armstrong and Okun, 2020). These compounds promote locomotion by activating D_2 receptors at striatal medium spiny neurons. Upon ligand binding, D_2 receptors primarily signal by recruiting $G_{i/o}$ proteins, which inhibit the activity of adenylate cyclase (Neve et al., 2004). G protein signaling is primarily terminated by the receptor interaction with β-arrestins, favoring receptor desensitization and internalization, together with promoting β-arrestin-dependent signaling (Lefkowitz and Shenoy, 2005). In the case of D_2 receptors, β-arrestin2-associated cell responses are mediated by the Akt-GSK3β pathway (Beaulieu et al., 2007). As such, D_2 receptors are capable of engaging in multimodal signal transduction patterns, similarly to what is reported for several G protein-coupled receptors (GPCRs) (Smith et al., 2018).

The property of receptor ligands to preferentially activate one signaling pathway over another - referred to as functional selectivity or signaling bias - has received considerable interest in molecular pharmacology, as it may present innovative therapeutic avenues (Whalen et al., 2011). Importantly, functional selectivity may harbor beneficial features in the context of PD. Transgenic mice lacking either the β-arrestin2 or the D_2 receptor gene share deficits in basal and cocaine- or amphetamine-induced locomotor activity, indicating the pivotal role of β-arrestin2 in regulating motor behavior (Baik et al., 1995; Beaulieu et al., 2005). Nevertheless, the viral expression of D_2 receptor mutants capable of exclusively signal through β-arrestin2 in the medium spiny neurons of D_2 receptor-knock-out mice failed to completely rescue the aforementioned motor deficits, indicating that both G protein and β-arrestin2 downstream pathways participate in concert to regulate locomotor behavior (Donthamsetti et al., 2020; Rose et al., 2018). Of note, the overexpression of β-arrestin2 was proven to mitigate the exacerbated G protein signaling associated with levodopa-induced dyskinesias in rats treated with 6-hydroxydopamine, in non-human primates exposed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), as well as in β-arrestin2 knock-out mice (Urs et al., 2015). For this reason, the characterization of the preferential β-arrestin2 signaling of dopaminergic agents used in antiparkinsonian therapies, and to a wider extent their signaling bias, appears of importance in treatment optimization.

In this context, it is important to consider that receptor functional bias is modulated by several biochemical determinants such as the availability of signaling partners or receptor density (Hermans, 2003). Indeed, varying dopamine receptor density has already been associated with the capacity to significantly influence the pharmacological profile of several GPCR ligands (Gazi et al., 1999; Koener et al., 2012). Nevertheless, even though the functional selectivity of the D_2 receptor has been extensively documented in several recombinant (Gay et al., 2004; Kilts et al., 2002; Laschet et

Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

al., 2019; Mottola et al., 2002) and murine models (Allen et al., 2011; Beaulieu et al., 2005; Urs et al., 2015, 2016), the putative influence of receptor density on the biased signaling of D_2 receptor ligands has not been addressed yet.

Single-photon emission computed tomography (SPECT) (Ichise et al., 1999; Verstappen et al., 2007) and positron emission tomography (PET) (Kaasinen et al., 2000; Rinne et al., 1995) studies have shown that the striatum of early and drug-naïve PD patients presented higher D_2 receptor-specific radioligand tracing as compared to control subjects, suggesting that the nigrostriatal neurodegeneration characterizing PD tends to be compensated by the up-regulation of D_2 receptors by striatal neurons. At variance, the reduced [^{11}C] raclopride binding observed in the striatum of PD patients treated for at least three years with levodopa (Antonini et al., 1997) or dopamine agonists (Politis et al., 2017) has linked chronic antiparkinsonian treatments with the striatal downregulation of D_2 receptors. These brain imaging studies constitute solid lines of evidence highlighting that the D_2 receptor density undergoes substantial changes in striatal medium spiny neurons of patients affected by PD, and so through the course of the disease and its treatment.

Thus, we hypothesized that altering the D_2 receptor density may drive significant changes in the signaling bias of antiparkinsonian drugs. In order to test this hypothesis, we employed a nanoluciferase-based complementation assay to monitor the recruitment of G_{i1} or β-arrestin2 proteins at the D_2 receptor long isoform (D_{2L}) in response to dopamine and a subset of clinically approved D_2 receptor agonists (pramipexole, ropinirole, and rotigotine; depicted in Fig. 1). This approach allows to examine of the recruitment of either G_{i1} or β-arrestin2 using a single receptor construct, limiting the possible interference of system bias on experimental observations. In addition, the assay has been performed in a cellular model enabling the standardized overexpression of the D_{2L} receptor through a tetracycline-inducible system. Combining these two approaches led to the obtention of a valuable model designed to indirectly characterize the influence of receptor density on the pharmacological profile of antiparkinsonian drugs.

2. Materials and methods

2.1. MATERIALS

Dopamine, pramipexole, ropinirole, X-tremeGene™ HP DNA transfection reagent, and DMSO were from Sigma-Aldrich (Diegem, Belgium). Rotigotine was from Adooq Bioscience (Irvine, CA). l-glutamine, penicillin/streptomycin, lipofectamine, and trypsin-EDTA, from Thermo Fisher Scientific (Waltham, MA). Cell culture medium (Dulbecco's Modified Eagle Medium), G418 (50 mg/mL Stock), and Hygromycin (50 mg/mL Stock) were from Invitrogen (Merelbeke, Belgium). 6-well and 96-well plates were from Greiner Bio-One (Wemmel, Belgium). Fetal bovine serum (FBS) was from Biowest (Riverside, MO). Doxycycline was from TakaraBio (Kusatsu, Japan). Coelenterazine-h was from Regis Technologies (Morton Grove, IL).

Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

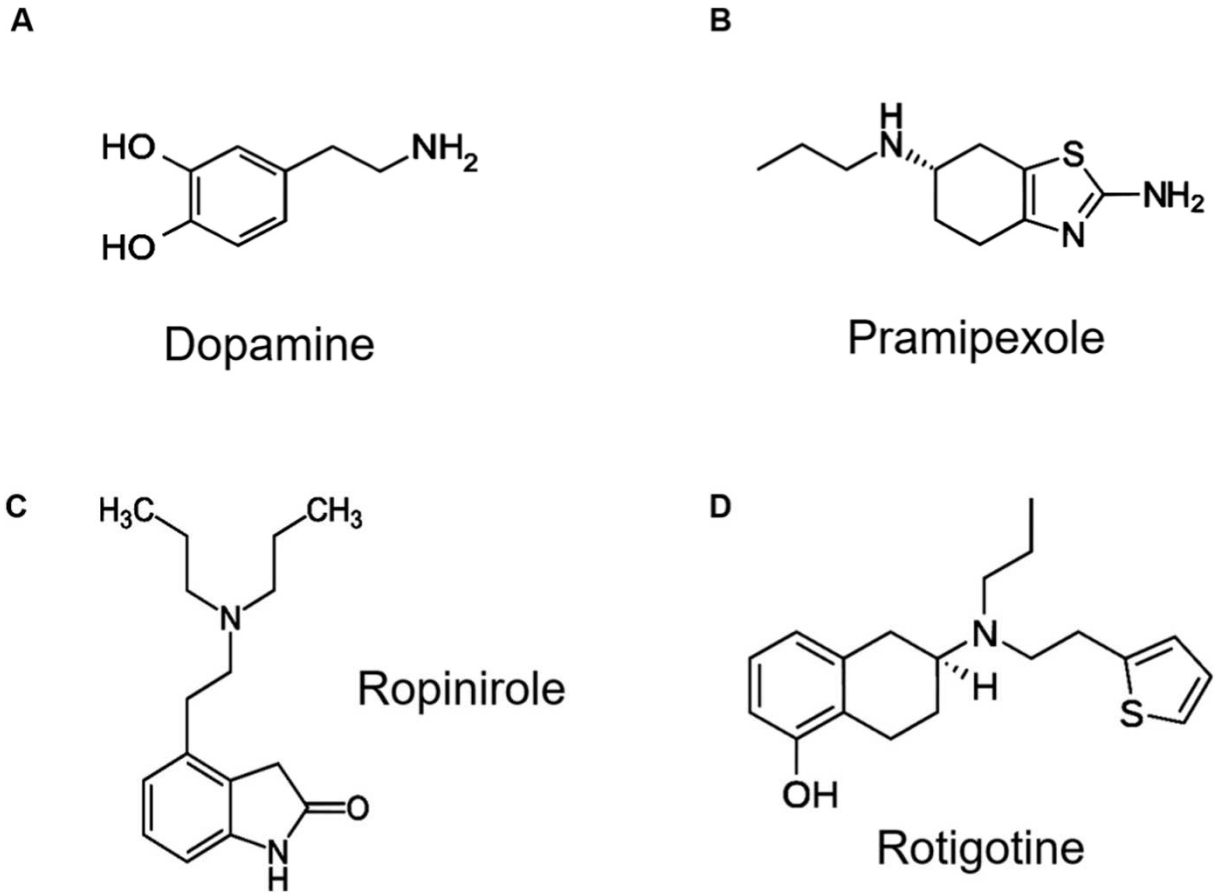

Fig. 1. Chemical structures of the compounds tested in the hereby presented study. (A) Dopamine. (B) Pramipexole. (C) Ropinirole. (D) Rotigotine.

2.2. MOLECULAR CLONING

D_{2L}-NP, G_{i1}-LgBiT, and β-arrestin2-LgBiT constructs were developed as described by Laschet et al. (2019). In brief, a small fragment of the nanoluciferase enzyme, referred to as natural peptide (NP) was fused at the C terminus of the D_{2L} receptor sequence, preceded by a flexible linker, yielding D_{2L}-NP construct. A Flag epitope was added at the N-terminus of the D_{2L} sequence. The G_{i1}-LgBiT and β-arrestin2-LgBiT constructs were obtained by cloning the sequences coding for G_{i1} alpha subunit or β-arrestin2 downstream of the large subunit of the nanoLuciferase enzyme (LgBiT), separated by a flexible linker. An HA epitope was added at the C-terminus of the G_{i1} sequence.

2.3. CELL CULTURE AND TRANSFECTION

HeLa Tet-On cells were stably transfected with a pTRE construct carrying the D_{2L} cDNA sequence using Lipofectamine™ and selected with G418 (500 µg/mL) and hygromycin (100 µg/mL), resulting in the obtention of a cell line referred to as HeLa Tet-On D_{2L} cells. The experiments were performed on a single clonal population purposely chosen for its low basal [^{3}H]spiperone binding in the absence of doxycycline, as specified by Koener et al. (2012). HeLa Tet-On D_{2L} and non transfected HeLa cells were routinely cultivated at 37 °C with 5% CO_2 in Dulbecco's modified Eagle medium supplemented

Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

with 1% l-glutamine, 1% penicillin/streptomycin, 10% FBS. HeLa Tet-On D_{2L} medium also included G418 (250 µg/mL) and hygromycin (50 µg/mL). Forty-eight hours prior to nanoluciferase complementation experiments, cells were plated in 6-wells plates at a density of 2 million cells/well in serum- and antibiotic-free medium. Four hours after plating, cells were co-transfected with the D_{2L}-NP and either the G_{i1}-LgBiT or the β-arrestin2-LgBiT plasmids at a 1:1 mass ratio using the X-tremeGene™ HP DNA transfection reagent with a reagent/DNA ratio of 3:1, according to the manufacturer's recommendations. After 24 h, the cell culture medium was replaced with a 10% FBS-enriched medium. When indicated, D_{2L} receptor expression was obtained by exposing cells for 24 h to 3 µg/mL of doxycycline.

2.4. MEASUREMENT OF G_{I1} OR B-ARRESTIN2 RECRUITMENT AT THE D_{2L} RECEPTOR WITH THE NANOLUCIFERASE COMPLEMENTATION ASSAY

Forty-eight hours post-transfection, cells grown in the presence or the absence of doxycycline were harvested and resuspended in Hank's balanced salt solution (HBSS) (NaCl 120 mM, KCl 5.4 mM, $MgSO_4$ 0.8 mM, and HEPES 10 mM) supplemented with 0.05 mM $Na_2S_2O_5$. Living cells were then distributed in 96-well plates, at a density of 50'000 cells/well in a volume of 80 µL and exposed to the indicated ligands (10 µL) or to vehicle solution (HBSS, 8% DMSO). The nanoluciferase bioluminescent signal was acquired using the Topcount® luminescence counter (PerkinElmer, Waltham, MA) after incubating cells for 15 min with 10 µL of a 10 µM coelenterazine-h solution (Regis Technologies, Morton Grove, IL).

2.5. DATA ANALYSIS

The bioluminescent signals from nanoluciferase complementation assays were quantified in relative light units (RLUs) as means ± SEM and expressed as a percentage above the basal signal, obtained from vehicle-treated cells, to control for variations in receptor expression. Data points represent the mean ± SEM of five independent experiments, performed in triplicates. For the different compounds tested, non-linear analyses of sigmoidal concentration-response curve fitting (fixed slope) were performed in order to determine their efficacy (E_{max}, expressed as a percentage of response above basal) and potency (pEC_{50}). Statistical analyses were performed using Graph Pad Prism, version 5.03 (GraphPad Software, CA). Experimental readouts from different experimental conditions were compared using unpaired, two-tailed Student's t-tests when comparing two data sets, while one-way ANOVA tests were conducted when comparing more than two data sets. Statistical differences from control conditions were assessed by applying Dunnett's post hoc test to One-way ANOVA statistical tests. Two-factors analyses were performed using the two-way ANOVA statistical test, followed by the Šídák post hoc test. When not mentioned within the text, the detailed reporting of the performed statistical tests can be found in the tables and their legend.

Bias factors were calculated as proposed by Kenakin et al. (2012). We first calculated the transduction coefficients ($Log(E_{max}/EC_{50})$) of each ligand by deriving E_{max} and EC_{50} values from the concentration-response curves obtained when assessing G_{i1}-LgBiT and β-arrestin2-LgBiT recruitment at the D_2-NP receptor, and so at both 0 and 3 µg/mL of doxycycline. Then, the

Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

transduction coefficients of D_2 receptor agonists were compared with the transduction coefficient of dopamine obtained for the same signaling pathway at 0 µg/mL of doxycycline, resulting in ΔLog (E_{max}/EC_{50}) values (I). Finally, the bias factors of the selected D_2 receptor ligands were obtained by comparing the ΔLog (E_{max}/EC_{50}) values related to G_{i1} and β-arrestin2 recruitment at the receptor mediated by each studied substance (II)

$$\Delta Log(E_{max}/EC_{50})_{Ligand} = \text{Log}(E_{max}/EC_{50})_{Ligand} - \text{Log}(E_{max}/EC_{50})_{Dopamine} \text{ (I)}$$

$$\Delta\Delta\text{Log}(E_{max}/EC_{50})_{Ligand} = \Delta\text{Log}(E_{max}/EC_{50})_{Ligand,\ Gi1} - \text{Log}(E_{max}/EC_{50})_{Ligand,\beta\text{-arrestin2}} \text{ (II)}$$

3. Results

3.1. DOXYCYCLINE-DEPENDENT D2L RECEPTOR OVEREXPRESSION

In order to circumvent the variability in protein expression level associated with transient transfection, we used a stably transfected cell line carrying an inducible system offering the possibility to control the expression of the unmodified and full-length D2L receptor after addition of doxycycline to the culture medium. This model was obtained by introducing the sequence coding for the D2L receptor in the Tet-On system harbored by HeLa cells, resulting in a cell population referred to as HeLa Tet-On D2L cells. The expression of the D_{2L} receptor in these cells was previously validated in [^{3}H]spiperone binding assays by Koener et al. (2012). Membrane homogenates derived from HeLa Tet-On D_{2L} cells exposed for 24 h to 3 µg/mL of doxycycline showed a 10-fold increase in the specific binding of the D_{2L} receptor radioligand (11,029 ± 834 cpm/mg of protein) when compared to homogenates not exposed to the doxycycline (1036 ± 65 cpm/mg of protein).

3.2. MONITORING OF LIGAND-EVOKED GI1 AND B-ARRESTIN2 RECRUITMENT AT THE D2L RECEPTOR

The constitutive and ligand-biased signaling at the D_{2L} receptor was assessed by the means of a split nanoluciferase complementation assay based on the NanoBiT® technology. This approach was first optimized by Laschet et al. (2019) for the specific monitoring of G proteins recruitment at the D_{2L} receptor, and further adapted to detect β-arrestin2 recruitment. The amino acid sequences of both signal transducers were fused with the large fragment of the nanoluciferase enzyme (LgBiT) at their N-terminus whereas the native peptide sequence of the small fragment of the enzyme (referred to as NP) was fused at the C-terminal of the D_{2L} receptor. The recruitment of G_{i1} and β-arrestin2 at the D_{2L}-NP receptor induced by dopamine and a subset of clinically relevant antiparkinsonian agents (pramipexole, ropinirole, and rotigotine) was therefore profiled by performing nanoluciferase complementation assays on HeLa Tet-On D_{2L} cells co-transfected with the D_{2L}-NP plasmid and either the G_{i1}-LgBiT or β-arrestin2-LgBiT constructs.

First, the recruitment of either G_{i1} or β-arrestin2 was examined in cells grown in the absence of doxycycline, and therefore without overexpression of the unlabeled D_{2L} receptor. In these

Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

conditions, the dopamine-evoked recruitment of G_{i1} (E_{max}: 16.08 ± 1.72, pEC_{50}: 8.05 ± 0.39) and β-arrestin2 (E_{max}: 17.71 ± 1.44, pEC_{50}: 7.60 ± 0.28) at the D_{2L}-NP receptor were similar (unpaired, two-tailed Student's t-test; extensive statistical reporting is shown in Table 1) (Fig. 2 A). Pramipexole promoted G_{i1} recruitment 575-fold more potently than β-arrestin2 (2.76 difference in pEC_{50} values) (Fig. 2 B, Table 1). Ropinirole, on the other hand, was capable of eliciting G_{i1} recruitment (E_{max}: 21.14 ± 2.33, pEC_{50}: 8.85 ± 0.32) while failing at inducing any detectable interaction between the D_{2L}-NP receptor and β-arrestin2-LgBiT proteins above the basal level (E_{max}: 4.41 ± 1.59, pEC_{50}: N/A), an observation that could be interpreted as a robust G_{i1}-bias (Fig. 2 C, Table 1). On the other hand, rotigotine appeared more prone to promote β-arrestin2 recruitment as compared to G_{i1}, both in terms of potency and efficacy, positioning rotigotine as a β-arrestin2-biased ligand (Fig. 2 D, Table 1).

Table 1. Summary of efficacy (E_{max}), potency (pEC_{50}), and bias of the tested D_{2L} receptor agonists at 0 µg/mL of doxycycline. The unpaired, two-tailed Student's t-test enabled to calculate the p-value of the differences in efficacy and potency measured for G_{i1} and β-arrestin2 recruitment at the D_{2L} receptor, enabling the estimation of recruitment bias.

Substance	**E_{max} ± SEM**		**Student's t-test (Unpaired, two-tailed)**		**pEC_{50} ± SEM**		**Student's t-test (Unpaired, two-tailed)**		**Bias**
	G_{i1}	**β-arrestin 2**	**$t_{(8)}$**	**p-value**	**G_{i1}**	**β-arrestin 2**	**$t_{(8)}$**	**p-value**	
Dopamine	16.08 ± 1.72	17.71 ± 1.44	0.73	0,49	8.05 ± 0.39	7.60 ± 0.28	0.94	0.38	Balanced
Pramipexole	21.86 ± 2.12	17.43 ± 3.55	1.07	0.32	9.88 ± 0.34	7.12 ± 0.38	5.41	0.006	G_{i1}
Ropinirole	21.14 ± 2.33	−4.41 ± 1.59	9.07	<0.0001	8.85 ± 0.32	N/A	27.66	<0.0001	G_{i1}
Rotigotine	26.01 ± 2.03	33.77 ± 1.97	2.74	0.02	9.82 ± 0.30	10.91 ± 0.22	2.930	0.02	β-arrestin 2

Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

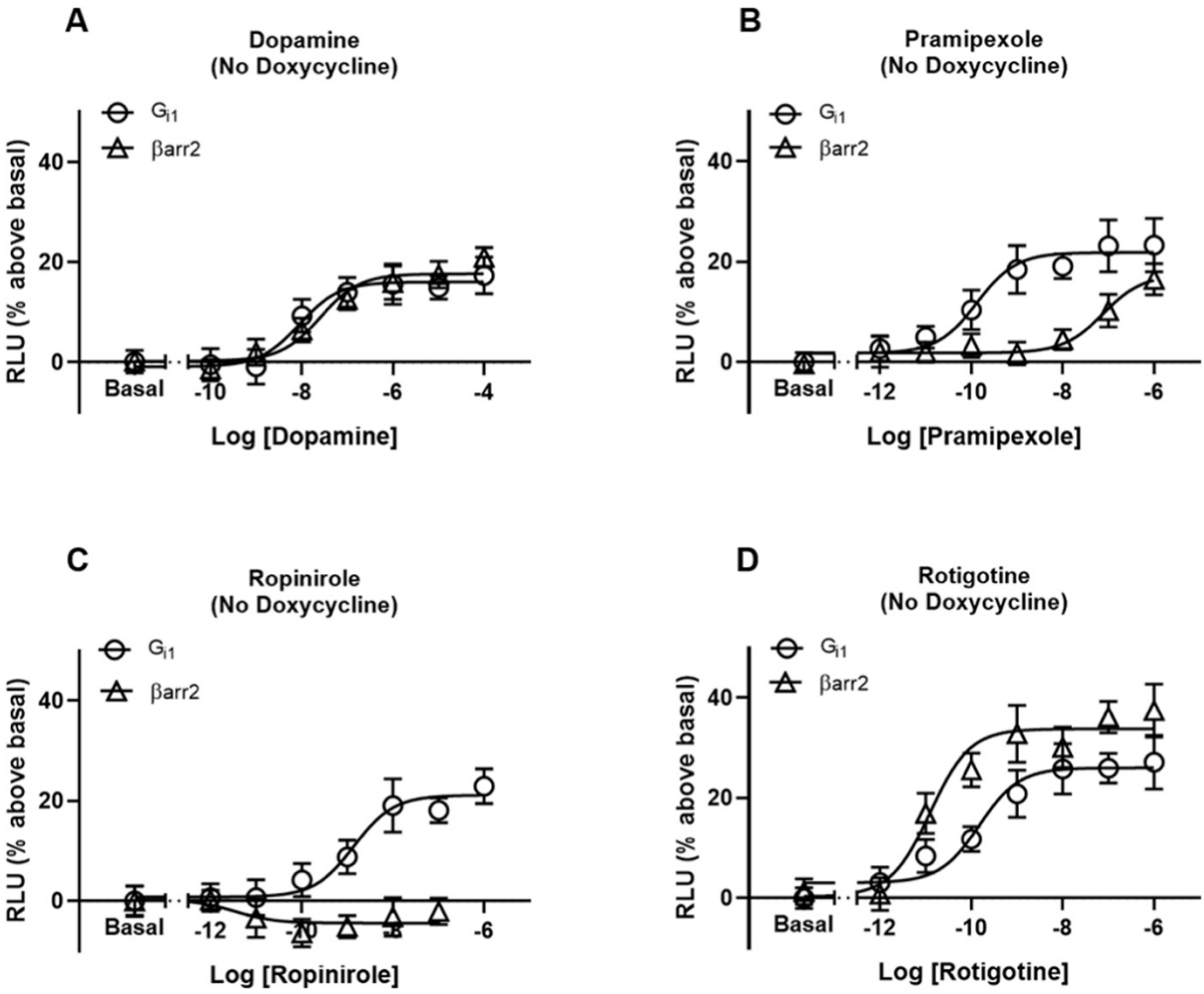

Fig. 2. G_{i1} and β-arrestin2 recruitment at the D_{2L} receptor induced by selected antiparkinsonian compounds. Concentration-response curves were obtained when measuring the interactions between the D_{2L}-NP receptor and the G_{i1}-LgBiT (G_{i1}) or β-arrestin2-LgBiT (βarr2) in response to dopamine (A), pramipexole (B), ropinirole (C), and rotigotine (D). Nanoluciferase complementation assays were performed on HeLa Tet-On D_{2L} cells not exposed to doxycycline.

Referring to the efficacy of the endogenous agonist dopamine, all the antiparkinsonian drugs tested for G_{i1} recruitment presented the pharmacological profile of full agonist (Fig. 3 A). Statistical analysis (one-way ANOVA followed by a Dunnett post hoc test; detailed statistical report on Table 2) revealed a significantly higher efficacy for rotigotine as compared to the other tested antiparkinsonian drugs (Fig. 3 B). This analysis also revealed that pramipexole and rotigotine, but not ropinirole, were more potent at promoting G_{i1} recruitment as compared to dopamine (Fig. 3 C). When considering the interactions between β-arrestin2 and the D_{2L}-NP receptor, dopamine and pramipexole exerted statistically comparable effects while ropinirole presented no detectable activity (Fig. 3D–F). Of note, rotigotine was capable of evoking the most potent and efficient responses amongst the tested compounds (Fig. 3D–F).

Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

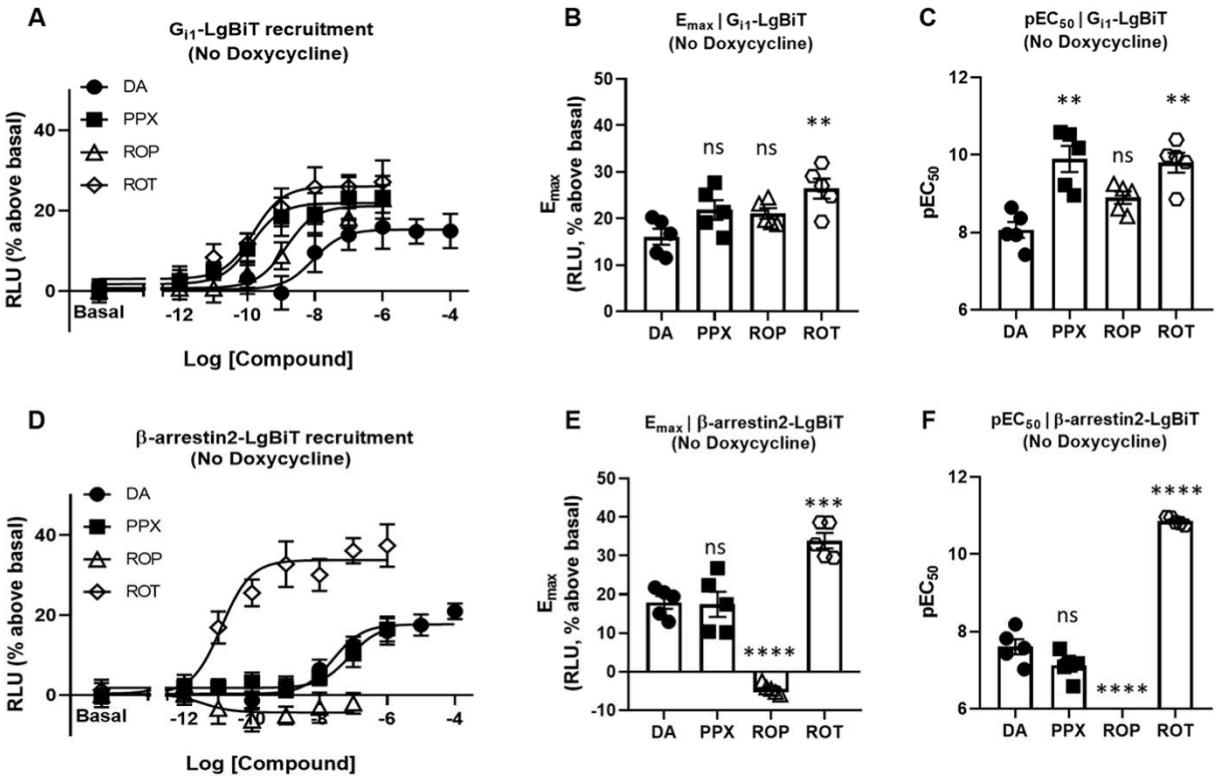

Fig. 3. Analysis of the pharmacological profile of dopamine (DA), pramipexole (PPX), ropinirole (ROP), and rotigotine (ROT) for G_{i1} and β-arrestin2 recruitment. (A, D) Characterization of the concentration-response curves obtained for the selected compounds when measuring protein-protein interactions between the D_{2L}-NP receptor and G_{i1}-LgBiT (A) or β-arrestin2-LgBiT (D). Nanoluciferase complementation assays were performed on HeLa Tet-On D_{2L} cells not exposed to doxycycline. Comparison of the efficacies (B, E) (Emax) and potencies (C, F) of the tested compounds for G_{i1}-LgBiT (B, C) or β-arrestin2-LgBiT (E, F) recruitment at the D_{2L}-NP receptor. Values were derived from the Statistical analyses were performed using a one-way ANOVA statistical test followed by Dunnett's post hoc test (ns, $p > 0.05$; ***, $p < 0.001$; ****, $p < 0.0001$).

Table 2. Statistical reporting of E_{max} and pEC_{50} comparison, 0 µg/mL of doxycycline. The efficacies (E_{max}) and potencies (pEC_{50}) of the tested D_{2L} receptor agonists measured for G_{i1} or β-arrestin2 recruitment were compared through the one-way ANOVA statistical test, followed by Dunnett's post hoc test.

		Adjusted p-values		
	$F_{(3,16)}$	**Pramipexole**	**Ropinirole**	**Rotigotine**
G_{i1}				
E_{max}	3.906	0.154	0.233	0.010
pEC_{50}	6.603	0.004	0.261	0.005
β-arrestin2				
E_{max}	41.020	0.999	<0.001	<0.001
pEC_{50}	310.800	0.448	<0.001	<0.001

Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

3.3. OVEREXPRESSING THE D2L RECEPTOR RESHAPES THE PHARMACOLOGICAL PROFILE OF ANTIPARKINSONIAN DRUGS

The recruitment of G_{i1} and β-arrestin2 was examined in HeLa Tet-On D_{2L} cells that were exposed to 3 µg/mL of doxycycline for 24 h before the assay. As previously mentioned, such treatment permitted the overexpression of the unmodified D_{2L} receptor. In these experimental conditions, the efficacy and potency of dopamine for G_{i1} recruitment were significantly higher than those for β-arrestin2, indicating a preferential recruitment of G_{i1} when compared to what was observed on cells not exposed to doxycycline (Fig. 4 A, detailed statistical report on Table 3). Such an effect was not observed when performing the experiments on native HeLa cells lacking the Tet-On system, indicating that doxycycline treatment *per se* was without influence on the dopamine-evoked recruitment of signal transducers (Supplementary Material Fig. S1). Pramipexole was found to promote the recruitment of both signal transducers with comparable potencies, but with significantly higher efficacy for G_{i1} (Fig. 4 B, Table 3). Similarly, ropinirole also appeared as G_{i1}-biased in these experimental conditions, as it induced nanoluciferase complementation only with the G_{i1}-LgBiT construct (Fig. 4 C, Table 3). Albeit rotigotine induced the interaction of the D_{2L}-NP receptor with both G_{i1} and β-arrestin2 with comparable potency, a significantly higher E_{max} value was obtained when testing β-arrestin2, positioning the latter as a preferential signaling partner recruited in response to rotigotine when overexpressing the D_{2L} receptor (Fig. 4 D, Table 3).

Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

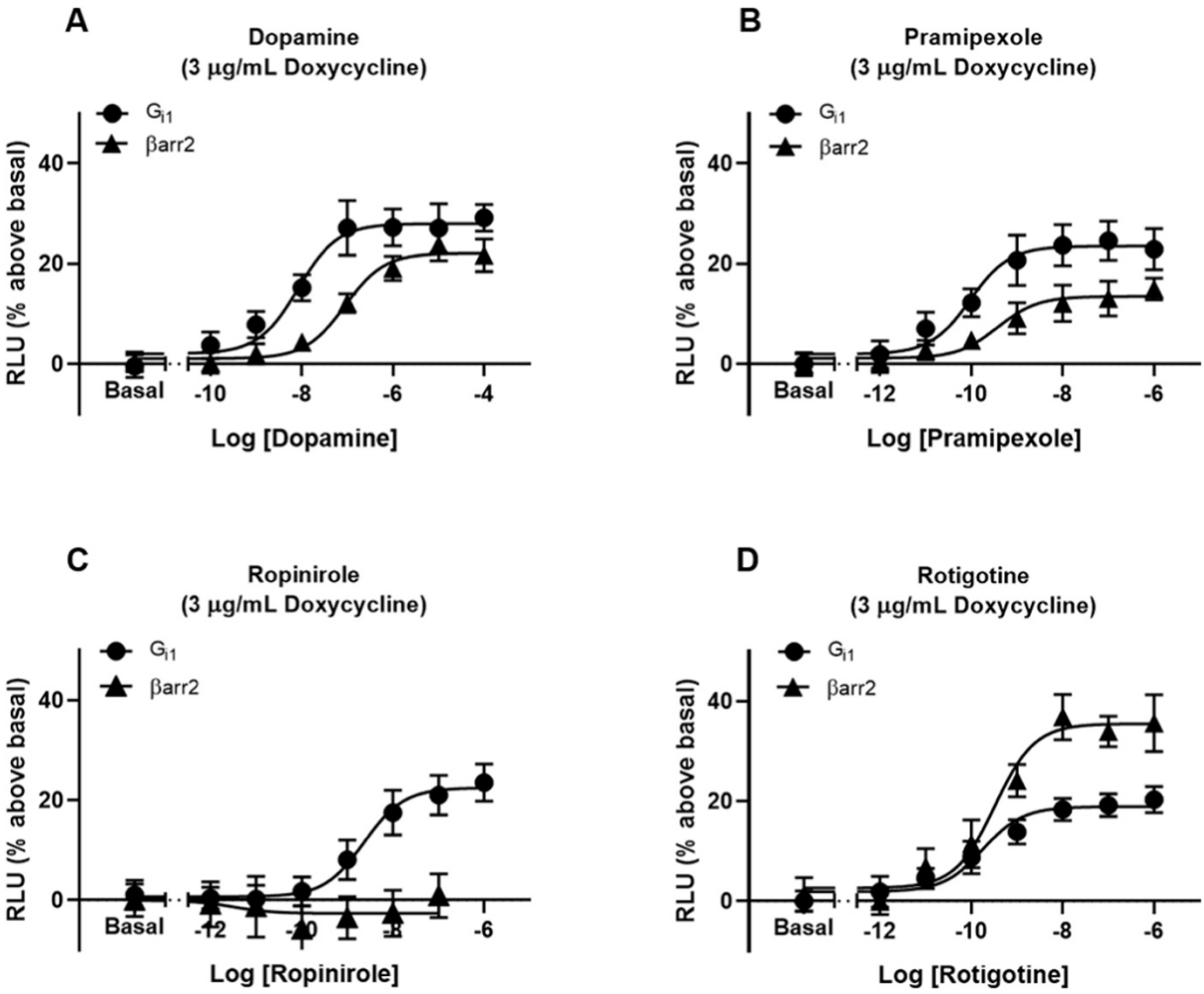

Fig. 4. G_{i1} and β-arrestin2 recruitment at the D_{2L} receptor induced by selected antiparkinsonian compounds after exposing cells to 3 µg/mL of doxycycline. Concentration-response curves were obtained when measuring the interactions between the D_{2L}-NP receptor and the G_{i1}-LgBiT (G_{i1}) or β-arrestin2-LgBiT (βarr2) in response to dopamine (A), pramipexole (B), ropinirole (C), and rotigotine (D). Nanoluciferase complementation assays were performed on HeLa Tet-On D_{2L} cells exposed for 24 h to 3 µg/mL of doxycycline.

Table 3. Summary of efficacy (E_{max}), potency (pEC_{50}) and bias of the tested D_{2L} receptor agonists at 3 µg/mL of doxycycline. The unpaired, two-tailed Student's t-test enabled to calculate the p-value of the differences in efficacy and potency measured for G_{i1} and β-arrestin 2 recruitment at the D_{2L} receptor, enabling the estimation of recruitment bias.

Substance	**E_{max} ± SEM**		**Student's t-test (Unpaired, two-tailed)**		**pEC_{50} ± SEM**		**Student's t-test (Unpaired, two-tailed)**		**Bias**
	G_{i1}	**β-arrestin 2**	**$t_{(8)}$**	**p-value**	**G_{i1}**	**β-arrestin 2**	**$t_{(8)}$**	**p-value**	

Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

Dopamine	27.98 ± 1.92	22.12 ± 1.59	2.35	0,046	8.08 ± 0.23	7.05 ± 0.20	3.379	0.0097	G_{i1}
Pramipexole	23.51 ± 1.93	17.43 ± 3.55	4.077	0.0036	10.03 ± 0.31	9.42 ± 0.46	1.100	0.3035	G_{i1}
Ropinirole	22.48 ± 2.27	−2.65 ± 2.32	9.903	<0.0001	8.63 ± 0.36	N/A	23.97	<0.0001	G_{i1}
Rotigotine	18.93 ± 1.51	35.46 ± 2.41	5.812	0.004	9.74 ± 0.29	9.49 ± 0.26	0.642	0.54	β-arrestin 2

When comparing the pharmacological profiles of the studied substances for G_{i1} recruitment, we noticed that at a high D_{2L} receptor density, both pramipexole and ropinirole shared an efficacy comparable to dopamine, while rotigotine behaved as a partial agonist. In these conditions, pramipexole and rotigotine, but not ropinirole, exhibited significantly higher potencies with respect to dopamine (Fig. 5 A-C; detailed statistical report on Table 4). Extending such analysis to β-arrestin2 revealed that a high receptor density is not sufficient to evoke ropinirole-induced interactions of the D_{2L} receptor with β-arrestin2. Nevertheless, such experimental conditions were characterized by the potent agonism of rotigotine. Of note, exposing HeLa Tet-On D_{2L} cells to 3 µg/mL of doxycycline for 24 h promoted the potent partial agonism of pramipexole for β-arrestin2 recruitment (Fig. 5D–F).

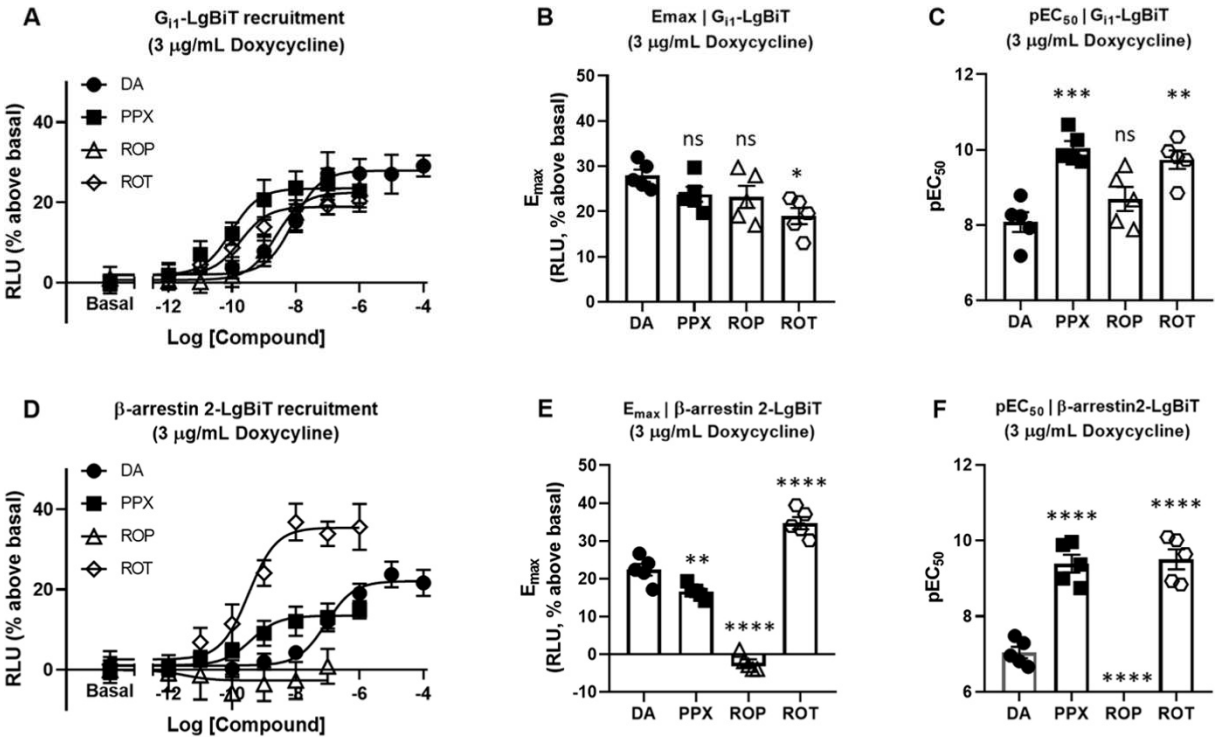

Fig. 5. Analysis of the pharmacological profile of dopamine (DA), pramipexole (PPX), ropinirole (ROP), and rotigotine (ROT) for G_{i1} and β-arrestin2 recruitment at the D_{2L} receptor after exposing cells to 3 µg/mL of doxycycline. (A, D) Characterization of the concentration-response curves obtained for the selected

Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

compounds when measuring protein-protein interactions between the D_{2L}-NP receptor and G_{i1}-LgBiT (A) or β-arrestin2-LgBiT (D). Nanoluciferase complementation assays were performed on HeLa Tet-On D_{2L} cells exposed for 24 h to 3 µg/mL of doxycycline, inducing the overexpression of the unlabeled, native D_{2L} receptor. Comparison of the efficacies (B, E) (E_{max}) and potencies (C, F) of the tested compounds for G_{i1}-LgBiT (B, C) or β-arrestin2-LgBiT (E, F) recruitment at the D_{2L}-NP receptor. Statistical analyses were performed using a one-way ANOVA statistical test followed by Dunnett's post hoc test (ns, $p > 0.05$; ***, $p < 0.001$; ****, $p < 0.0001$).

Table 4. Statistical reporting of E_{max} and pEC_{50} comparison, 3 µg/mL of doxycycline. The efficacies (E_{max}) and potencies (pEC_{50}) of the tested D_{2L} receptor agonists measured for G_{i1} or β-arrestin2 recruitment at the D_{2L} receptor were compared through the one-way ANOVA statistical test, followed by Dunnett's post hoc test.

		Adjusted p-values		
	$F_{(3,16)}$	**Pramipexole**	**Ropinirole**	**Rotigotine**
G_{i1}				
E_{max}	3.746	0.271	0.145	0.012
pEC_{50}	9.298	0.001	0.452	0.004
β-arrestin 2				
E_{max}	83.620	0.005	<0.001	<0.001
pEC_{50}	250.800	<0.001	<0.001	<0.001

3.4. CHANGING D_{2L} RECEPTOR DENSITY HAS DISTINCT IMPACTS ON THE RECRUITMENT OF SIGNAL TRANSDUCERS IN RESPONSE TO ANTIPARKINSONIAN DRUGS

The changes in the functional selectivity of D_2 receptor ligands caused by the doxycycline-induced D_{2L} receptor overexpression were appraised by distinctly comparing the differences in their efficacy and potency to recruit G_{i1} and β-arrestin2 at the D_{2L}-NP receptor after exposing the cells to doxycycline or not. The two-way ANOVA statistical test followed by Šídák multiple comparisons highlighted that the high receptor density increased the apparent efficacy of G_{i1} recruitment over β-arrestin2 for both dopamine and pramipexole (Fig. 6 A; $F_{(1,32)} = 2.287$, adjusted p-value: 0.001 and < 0.021, respectively). Conversely, a higher D_{2L} receptor density further enhanced rotigotine E_{max} for inducing β-arrestin2 interactions with the D_{2L}-NP receptor (Fig. 6 B; $F_{(1,32)} = 2.287$, adjusted p-value: <0.001). Increasing D_{2L} receptor density did not affect the potency of dopamine with respect of the two tested signaling partners (Fig. 6 B; $F_{(1,32)} = 2.287$, adjusted p-value: 0.142). In the absence of doxycycline exposure, pramipexole evoked the recruitment of G_{i1} with a higher potency than for β-arrestin2, while β-arrestin2 was recruited more potently than G_{i1} in response to rotigotine stimulation (Fig. 6 B). However, D_{2L} receptor overexpression caused both ligands to recruit the two transducers with comparable potencies (Fig. 6 B; $F_{(1,32)} = 2.287$ adjusted p-value: <0.001 for both

Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

drugs). Of note, doxycycline treatment was without effect on the G_{i1} recruitment elicited by ropinirole (Fig. 6 A and B; $F_{(1,32)} = 2.287$, adjusted p-values: 0.925 for efficacy and 0.878 for potency).

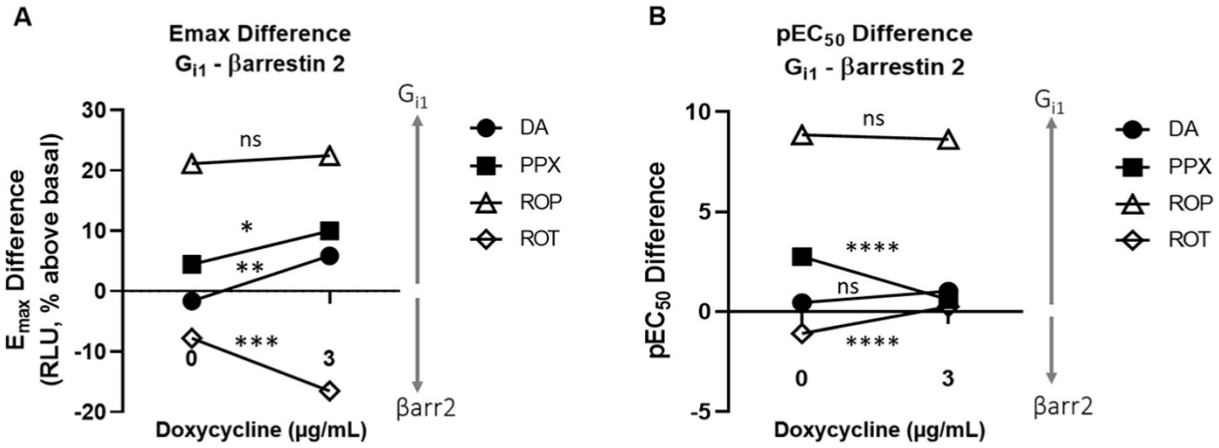

Fig. 6. Receptor density-induced variations in the preferential recruitment of G_{i1} or β-arrestin2 in response to selected D_{2L} receptor agonists. The preferential recruitment profile of dopamine (DA), pramipexole (PPX), ropinirole (ROP), and rotigotine (ROT) was estimated by subtracting the efficacy (A) and potency (B) values measured for β-arrestin2-LgBiT from those relative to G_{i1}-LgBiT recruitment at the D_{2L}-NP receptor in the absence and in the presence of receptor overexpression (respectively corresponding to 0 and 3 µg/mL of doxycycline treatment). Positive values highlight a stronger efficacy or potency for G_{i1} over β-arrestin2 (βarr2) recruitment, while negative values indicate a stronger efficacy or potency for β-arrestin2 (βarr2) over G_{i1} recruitment. Statistical analyses were performed using a two-way ANOVA statistical test followed by Šídák multiple comparison post hoc test (ns, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$).

4. Discussion

First-line treatments for PD frequently consist in the administration of levodopa or D_2 receptor agonists such as pramipexole, ropinirole, and rotigotine (Armstrong and Okun, 2020). However, the expression of the D_{2L} receptor is subject to fluctuations during the different stages of the disease (Ichise et al., 1999; Kaasinen et al., 2000; Rinne et al., 1995; Verstappen et al., 2007) and its treatment (Antonini et al., 1997; Politis et al., 2017). Receptor density has been described as a key determinant of experimental outcome in several pharmacological investigations (Hermans et al., 1999; Jarvis and Thompson, 2019; Kenakin, 1997, 2013), especially when characterizing dopaminergic ligands (Gazi et al., 1999; Watts et al., 1995). For instance, Koener et al. have observed that the partial agonism at the D_{2L} receptor of the antipsychotic drug aripiprazole could be validated only when expressing the receptor at a very high density (Koener et al., 2012). In addition, receptor density has also been quoted as a putative driver of the altered receptor signaling in response to drugs, also referred to as functional selectivity or signaling bias, both in vitro and in vivo (Singleton et al., 2021). For these reasons, we hypothesized that manipulating the D_{2L} receptor density could influence the signaling bias of D_{2L} receptor agonists prescribed as treatment of PD.

We herein report on the monitoring of agonist-induced G_{i1} and β-arrestin2 recruitment at the D_{2L} receptor with a functional complementation assay based on the NanoBiT® technology. This split

Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

nanoluciferase-based assay was successfully adapted for the screening of a panel of D_{2L} receptor ligands by Laschet et al. (2019). By translating protein-protein interactions in a quantifiable luminescent signal, the complementation assay enables to accurately assess early molecular responses in the signaling cascades, bypassing the molecular crosstalk thwarting the interpretation of readouts from downstream outcomes, such as the accumulation of second messengers. Moreover, the nanoluciferase complementation assay offers a more sensitive option to other methodologies for the measurement of protein-protein interactions, notably bioluminescence and fluorescence resonance energy transfer (BRET and FRET, respectively), and presents reversibility features absent with other protein complementation assays (PCA) (Dixon et al., 2016; Laschet et al., 2019; Laschet and Hanson, 2021). The hereby presented system also offers the advantage of measuring receptor interactions with β-arrestin2 and G proteins with an identical receptor construct, limiting the possible interference of system bias commonly observed when employing distinct assays to measure receptor interactions with signal transducers (for instance arrestin recruitment in combination with cAMP accumulation).

With the goal of assessing the preferential signaling triggered by antiparkinsonian compounds at two distinct D_{2L} receptor densities, nanoluciferase complementation assays were performed in a cellular model in which the expression of unmodified D_{2L} receptor could be manipulated. Importantly, this unmodified D_{2L} receptor is unable to participate in the luminescent readout. Thus, in this experimental paradigm, the nanoluciferase-based biosensor was used as a pharmacological probe for the signaling bias of D_{2L} receptor ligands.

In the absence of doxycycline-mediated D_{2L} receptor overexpression, dopamine was proven to recruit both G_{i1} and β-arrestin2 in a comparable fashion, whereas pramipexole displayed a significant orientation towards G_{i1} recruitment. Conversely, rotigotine elicited a substantial recruitment of β-arrestin2. However, increasing D_{2L} receptor densities caused dopamine-evoked recruitment of signaling transducers to evolve towards a G_{i1} bias. On the other hand, both pramipexole and rotigotine maintained their respective biases for G_{i1} and β-arrestin2, albeit with a reduction in amplitude, indicating a slightly more balanced pharmacological profile at a higher D_{2L} receptor density. It is noteworthy to underscore that stimulating D_{2L} receptors with ropinirole did not result in any detectable β-arrestin2 recruitment, both at low and high receptor density, highlighting a marked recruitment bias for G_{i1} which was unaffected by receptor density.

The G_{i1} biases of pramipexole and ropinirole observed in this study are consistent with previous reports obtained from either split nanoluciferase assays in transfected HEK293 cells (Laschet et al., 2019) or when combining the measurement of cyclic AMP accumulation with the PathHunter β-arrestin2 assay in transfected Chinese hamster ovary (CHO) cells (Brust et al., 2015). Of note, both research works reported a weak but detectable activity of ropinirole with regard to β-arrestin2 recruitment, similarly to what was observed by Klewe et al. in HEK293 cells using BRET (Klewe et al., 2008). However, our findings are in sharp contrast with the observations of Mann et al., reporting the balanced profile of ropinirole through BRET experiments in HEK293 cells (Mann et al., 2021). Such discrepancy with our data with respect to ropinirole-induced β-arrestin2 recruitment may be associated with the existence of different reserve receptor pools across experimental setups, arising

Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

from distinct transfection efficiencies, potentially influencing the potency and efficacy of the tested compounds (Kenakin, 1997, 2013). Nevertheless, differences in assay sensitivity, together with the specific biochemical features distinguishing cellular models, such as membrane lipid composition, the nature and expression levels of scaffold or regulatory proteins (namely regulators of G protein signaling (RGS)) might also underpin considerable differences in the detected receptor coupling (Hermans, 2003).

Importantly, the presented data underscore that ligand bias is significantly influenced by receptor density, and that endogenous ligands commonly referred to as "balanced", such as dopamine, are indeed subject to variations in their signaling bias as a function of the dynamics characterizing the molecular environment in which they act. Thus, the notion of balanced agonism appears as being accompanied by a system-specific nuance. This observation is particularly relevant, considering that the signaling profile natural agonists is frequently used as a reference for the assessment of the signaling bias of other compounds (Gundry et al., 2017; Kenakin et al., 2012; Nagi and Pineyro, 2016; Smith et al., 2018).

Several authors express bias under the form of a unique value, derived from the integration of both efficacy and potency, referred to as transduction coefficient (Kenakin et al., 2012; Nagi and Pineyro, 2016). Ligands' transduction coefficients obtained for a single signaling partner are first compared with the transduction coefficient of a reference agonist (frequently the endogenous receptor ligand), and then compared across different signaling pathways, yielding a system-independent indicator of signaling bias (Kenakin et al., 2012; Nagi and Pineyro, 2016). This quantitative method of analysis has nevertheless been criticized as prompting a distorted interpretation of ligand properties (Onaran et al., 2017; Onaran and Costa, 2021). However, when applied to our data, appraising the bias factor between G_{i1} and β-arrestin2 led to conclusions that appeared consistent with our initial analysis that separately compared the differences in efficacies and potencies of the examined signal transducers. Indeed, the bias factor analysis did provide further support in evidencing the moderating effect that increasing receptor density exerted on the recruitment biases of pramipexole and rotigotine (Fig. 7; $F_{(1,32)} = 2.287$ adjusted p-values: 0.300 for dopamine, <0.001 for pramipexole and 0.036 for rotigotine). Obviously, since dopamine was used as reference, the bias factor approach does not allow to appreciate variations in the preferential signaling of dopamine when increasing the receptor density.

Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

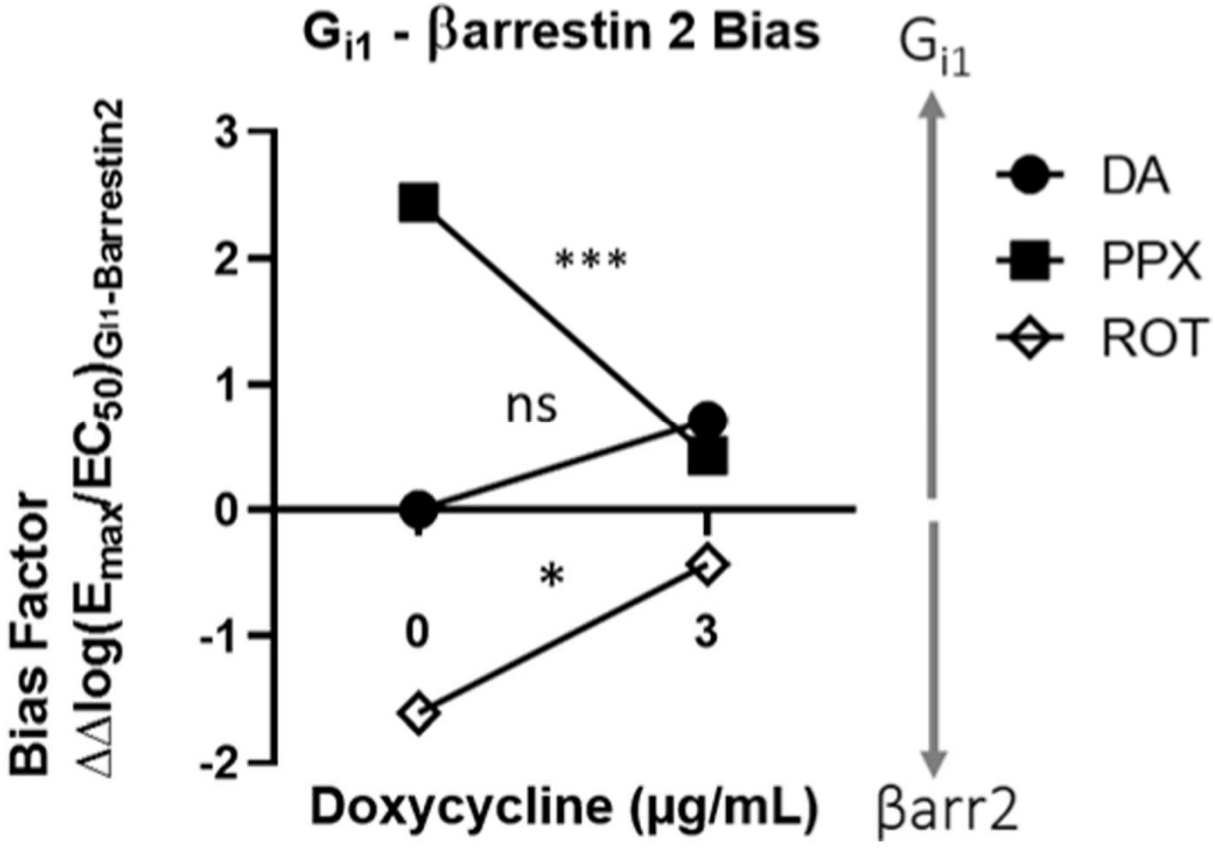

Fig. 7. Receptor density-induced variations in the preferential recruitment of G_{i1} or β-arrestin2 of selected D_{2L} receptor agonists when considering the bias factor. The preferential signaling of dopamine (DA), pramipexole (PPX), and rotigotine (ROT) were estimated by calculating the bias factor ($\Delta\Delta Log\ (E_{max}/EC_{50})_{Gi1\text{-}\beta arrestin2}$) for each compound when considering G_{i1}-LgBiT and β-arrestin2-LgBiT recruitment at the D_{2L}-NP receptor in the absence and in the presence of receptor overexpression (respectively corresponding to 0 and 3 µg/mL of doxycycline treatment). Ropinirole was excluded from the analysis as it displayed no detectable activity for β-arrestin2-LgBiT recruitment at the D_{2L}-NP receptor. The transduction coefficients ($Log\ (E_{max}/EC_{50})$) obtained from dopamine at 0 µg/mL of doxycycline were used as a reference value for the calculation of the bias factor. Positive values highlight G_{i1} bias, while negative values indicate β-arrestin2 (βarr2) bias. Statistical analyses were performed using a two-way ANOVA statistical test followed by Šídák multiple comparison post hoc test (ns, $p > 0.05$; *, $p < 0.05$; ***, $p < 0.001$).

In the context of PD, the activation of the β-arrestin2 pathway has been associated with beneficial outcomes, namely by promoting locomotion and by protecting against the disproportionate G protein signaling characterizing levodopa-induced dyskinesias (Urs et al., 2015). Hence, considering that our data describe rotigotine as the most β-arrestin2-biased ligand among the tested substances, it can be inferred that rotigotine would present a particularly beneficial therapeutic option for the treatment of both early and late PD when compared to pramipexole or ropinirole, in line with clinical research outcomes (Frampton, 2019; Giladi et al., 2016; Poewe et al., 2007; Raeder et al., 2021).

In this context, it is relevant to note that the β-arrestin2 biased ligand rotigotine presents a noticeable structural difference when compared to pramipexole and ropinirole, which are both associated with G protein-oriented recruitment selectivity (Fig. 1). Given the putative beneficial

Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

potential of β-arrestin2 bias in PD treatment, the exploration of the structure-activity relationship (SAR) focusing on the recruitment bias of dopamine receptor agonists might be of relevant therapeutic interest. Indeed, several SAR studies have unraveled the molecular determinants of biased signaling at the D_{2L} receptor in response to antipsychotic compounds (Chun et al., 2018; McCorvy et al., 2018; Shen et al., 2019; Shonberg et al., 2013; Von Moo et al., 2021). The extension of this approach to D_{2L} receptor agonists and to a series of synthetic analogues characterized by distinct chemical substitutions, as exemplified by Bonifazi et al. (Bonifazi et al., 2017, 2019), may facilitate the identification of the structural requirements for β arrestin2-oriented biased agonism. For instance, Stepniewski et al. have shown that ligands interaction with the histidine 6.55 residue within the sixth transmembrane helix of the D_2 receptor is essential for promoting β arrestin2 recruitment (Stepniewski et al., 2021). Moreover, the authors have highlighted that the dopamine analogue S-5-OH-DPAT presents a balanced, "dopamine-like" coupling profile (Stepniewski et al., 2021). Given that the structure of rotigotine is similar to S-5-OH-DPAT with the exception of a thiophen ring, it might be speculated that this chemical substituent behaves as a structural driver for enhanced β arrestin2 recruitment. Together, structural and functional studies should contribute to the development of a novel class of β-arrestin2-biased agonists of the D_{2L} receptor endowed with an optimized antiparkinsonian activity.

Nevertheless, β-arrestin2 biased ligands may not constitute the "silver bullet" therapeutic option in PD, as dopamine-induced locomotion appears as not entirely independent from the G proteins-downstream signaling (Donthamsetti et al., 2020; Rose et al., 2018). Indeed, clinical observations have reported rotigotine as being inferior to the G_{i1}-biased ligands pramipexole and ropinirole when considering the long-term efficacy at treating PD motor symptoms (Frampton, 2019; Giladi et al., 2007; Zhuo et al., 2017). These lines of evidence may be explained by a particularly sustained phenomenon of receptor internalization associated with the β-arrestin2 bias of rotigotine. Of note, the same clinical reports presented rotigotine as the most tolerable compound among the studied dopamine agonists, notably with regards to motor complications, which are associated with G_{i1} bias (Urs et al., 2015). These considerations might highlight the potential benefit of a balanced agonism profile in late PD, providing molecular support for the common practice of prescribing levodopa at advanced stages of the disease (Gray et al., 2014; Zahoor et al., 2018).

Notwithstanding, it is important to state that our interpretation arises from experiments performed on recombinant cell lines. In a more clinically-oriented framework, the multifaceted aspects of the tested compounds with respect to dopaminergic pharmacology as a whole shall not be overlooked, especially as the examined dopamine agonists have a remarkable affinity for dopamine receptors other than D_2 (Eden et al., 1991; Mierau et al., 1995; Wood et al., 2015). Considering our findings from a more holistic perspective might also yield insights of relevant interest in neuropharmacology. In detail, as receptor density has been shown to drive the signaling bias of receptor ligands, it can be assumed that distinct dopamine receptor densities across brain structures (Papenberg et al., 2019) may underpin region-dependent functional responses to dopamine, but also to antiparkinsonian and antipsychotic compounds.

Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

Of note, our results may not entirely be explained by receptor density alone, and the putative contribution of a plurality of other molecular mechanisms shall not be overlooked. For instance, the expression level of G protein-coupled receptor kinase (GRK) 2 in our cellular model may play a relevant role in determining the functional readouts of the tested compounds. Indeed, distinct expression levels of GRK2 between striatal and cortical tissues have been associated with opposite pharmacological profiles in response to UNC9994 (Urs et al., 2016). Since GRK2 is capable of promoting the ligand-induced recruitment of β-arrestin2 (Urs et al., 2016), one might speculate that increased levels of GRK2 expression levels would facilitate the β-arrestin2 recruitment in response to all compounds, including ropinirole.

Moreover, the observed recruitment biases might be explained by the competition dynamics between G_{i1} and β-arrestin2 for the same pool of receptors, which would be influenced by their different stoichiometry (Hermans, 2003) and kinetics of interactions with GPCRs (Beaulieu and Gainetdinov, 2011). In this context, another relevant factor possibly underlying density-driven changes in functional signaling may reside in D_2 homodimerization. Indeed, high receptor density of D_2 receptors has been associated with the tendency to promote their constitutive assembly (Ferraiolo et al., 2021; Wouters et al., 2019) promoting the establishment of efficient homodimeric signaling units (Han et al., 2009), and even of higher-order oligomers (Guo et al., 2008). Albeit the role of receptor density as a key factor in pharmacological operational models has been thoroughly established (Kenakin, 2009, 2013) the molecular underpinnings of such phenomena remain unraveled, and, given their therapeutic potential, may deserve further investigation.

5. Conclusion

In conclusion, this study provides evidence for the importance of receptor density in shaping the pharmacological profile of the D_{2L} agonists dopamine, pramipexole, and rotigotine with respect to their recruitment bias between G_{i1} and β-arrestin2. These compounds are commonly used in clinical practice, and since D_{2L} receptor density fluctuates during the progression and treatment of PD, the herein reported findings may be relevant for therapeutic optimization, not only in the context of PD but also for the restless leg syndrome (RLS). Indeed, this neurological disorder of movement control is associated with increased D_{2L} receptor expression in the striatum (Červenka et al., 2006), and is commonly treated with levodopa, pramipexole, ropinirole, or rotigotine (Winkelmann et al., 2018). Finally, the herein presented molecular investigation indicates the β-arrestin2 biased ligand rotigotine as potentially qualifying amongst the most beneficial D_2 receptor agonist for the treatment of both early and late PD, together with confirming levodopa as an efficient therapeutic option in later PD.

List of chemical compounds studied in the article

- **Dopamine:** 4-(2-Aminoethyl)benzene-1,2-diol

Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

- **Pramipexole:** (S)–N6-Propyl-4,5,6,7-tetrahydrobenzo [d]thiazole-2,6-diamine
- **Rotigotine:** (6S)-6-[propyl(2-thiophen-2-ylethyl)amino]-5,6,7,8-tetrahydronaphthalen-1-ol
- **Ropinirole:** 4-[2-(dipropylamino)etcomhyl]-1,3-dihydro-2H-indol-2-one

List of abbreviations

BRET bioluminescence resonance energy transfer

CHO Chinese hamster ovary

D_{2L} D_2 receptor long isoform

FBS fetal bovine serum

FRET fluorescence resonance energy transfer

GPCR G-protein-coupled receptor

HBSS Hank's balanced salt solution

HEK human embryonic kidney

MPTP 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

NanoBiT Nanoluciferase binary technology

NP natural peptide

PBS phosphate buffered saline

PCA protein complementation assays

PD Parkinson's Disease

PET positron emission tomography

RLU relative light units

RGS regulator of G protein signaling

RLS restless legs syndrome

SAR structure-activity relationship

SPECT single photon emission computed tomography

CRediT authorship contribution statement

Mattia Ferraiolo: Conceptualization, Formal analysis, Investigation, Writing – original draft, Visualization. **Hicham Atik:** Conceptualization, Formal analysis, Investigation, Visualization. **Romane Ponthot:** Formal analysis, Investigation, Visualization. Beryl Koener: Methodology,

Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

Investigation, Formal analysis. **Julien Hanson:** Methodology, Conceptualization, Supervision, paper revision. **Emmanuel Hermans:** Conceptualization, Resources, Writing – original draft, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

None.

6. Acknowledgements

This work was supported by the Fonds de la Recherche Scientifique – FNRS. M.F. is a research fellow of the FNRS. J.H. is senior research associate of the FNRS.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuropharm.2022.108942.

References

Allen, J.A., Yost, J.M., Setola, V., Chen, X., Sassano, M.F., Chen, M., Peterson, S., Yadav, P.N., Huang, X.P., Feng, B., Jensen, N.H., Che, X., Bai, X., Frye, S.V., Wetsel, W.C., Caron, M.G., Javitch, J.A., Roth, B.L., Jin, J., 2011. Discovery of β-arrestin-biased dopamine D 2 ligands for probing signal transduction pathways essential for antipsychotic efficacy. Proc. Natl. Acad. Sci. U.S.A. 108, 18488–18493. https://doi.org/10.1073/pnas.1104807108.

Antonini, A., Schwarz, J., Oertel, W.H., Pogarell, O., Leenders, K.L., 1997. Long-term changes of striatal dopamine D2 receptors in patients with Parkinson's disease: a study with positron emission tomography and [11C]raclopride. Mov. Disord. 12, 33–38. https://doi.org/10.1002/mds.870120107.

Armstrong, M.J., Okun, M.S., 2020. Diagnosis and treatment of Parkinson disease: a review. JAMA, J. Am. Med. Assoc. 323, 548–560. https://doi.org/10.1001/jama.2019.22360.

Baik, J.H., Picetti, R., Saiardi, A., Thiriet, G., Dierich, A., Depaulis, A., Le Meur, M., Borrelli, E., 1995. Parkinsonian-like locomotor impairment in mice lacking dopamine D2 receptors. Nature 377 (6548), 424–428. https://doi.org/10.1038/377424a0.

Beaulieu, J.M., Gainetdinov, R.R., 2011. The physiology, signaling, and pharmacology of dopamine receptors. Pharmacol. Rev. 63, 182–217. https://doi.org/10.1124/pr.110.002642.

Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

Beaulieu, J.M., Gainetdinov, R.R., Caron, M.G., 2007. The Akt-GSK-3 signaling cascade in the actions of dopamine. Trends Pharmacol. Sci. 28, 166–172. https://doi.org/10.1016/j.tips.2007.02.006.

Beaulieu, J.M., Sotnikova, T.D., Marion, S., Lefkowitz, R.J., Gainetdinov, R.R., Caron, M. G., 2005. An Akt/β-arrestin 2/PP2A signaling complex mediates dopaminergic neurotransmission and behavior. Cell 122, 261–273. https://doi.org/10.1016/j.cell.2005.05.012.

Bonifazi, A., Yano, H., Ellenberger, M.P., Muller, L., Kumar, V., Zou, M.F., Cai, N.S., Guerrero, A.M., Woods, A.S., Shi, L., Newman, A.H., 2017. Novel bivalent ligands based on the sumanirole pharmacophore reveal dopamine D2 receptor (D2R) biased agonism. J. Med. Chem. 60, 2890–2907. https://doi.org/10.1021/acs.jmedchem.6b01875.

Bonifazi, A., Yano, H., Guerrero, A.M., Kumar, V., Hoffman, A.F., Lupica, C.R., Shi, L., Newman, A.H., 2019. Novel and potent dopamine D2 receptor go-protein biased agonists. ACS Pharmacol. Transl. Sci. 2, 52–65. https://doi.org/10.1021/acsptsci.8b00060.

Brust, T.F., Hayes, M.P., Roman, D.L., Burris, K.D., Watts, V.J., 2015. Bias analyses of preclinical and clinical D2 dopamine ligands: studies with immediate and complex signaling pathways. J. Pharmacol. Exp. Therapeut. 352, 480–493. https://doi.org/10.1124/jpet.114.220293.

Cervenka, S., Pålhagen, S.E., Comley, R.A., Panagiotidis, G., Cselényi, Z., Matthews, J.C., Lai, R.Y., Halldin, C., Farde, L., 2006. Support for dopaminergic hypoactivity in restless legs syndrome: a PET study on D2-receptor binding. Brain 129, 2017–2028. https://doi.org/10.1093/brain/awl163.

Chun, L.S., Vekariya, R.H., Free, R.B., Li, Y., Lin, D.T., Su, P., Liu, F., Namkung, Y., Laporte, S.A., Moritz, A.E., Aubé, J., Frankowski, K.J., Sibley, D.R., 2018. Structure-activity investigation of a G protein-biased agonist reveals molecular determinants for biased signaling of the D2 dopamine receptor. Front. Synaptic Neurosci. 10, 2. https://doi.org/10.3389/FNSYN.2018.00002/BIBTEX.

Dixon, A.S., Schwinn, M.K., Hall, M.P., Zimmerman, K., Otto, P., Lubben, T.H., Butler, B. L., Binkowski, B.F., MacHleidt, T., Kirkland, T.A., Wood, M.G., Eggers, C.T., Encell, L.P., Wood, K.V., 2016. NanoLuc complementation reporter optimized for accurate measurement of protein interactions in cells. ACS Chem. Biol. 11, 400–408. https://doi.org/10.1021/acschembio.5b00753.

Donthamsetti, P., Gallo, E.F., Buck, D.C., Stahl, E.L., Zhu, Y., Lane, J.R., Bohn, L.M., Neve, K.A., Kellendonk, C., Javitch, J.A., 2020. Arrestin recruitment to dopamine D2 receptor mediates locomotion but not incentive motivation. Mol. Psychiatr. 25, 2086–2100. https://doi.org/10.1038/s41380-018-0212-4.

Eden, R.J., Costall, B., Domeney, A.M., Gerrard, P.A., Harvey, C.A., Kelly, M.E., Naylor, R.J., Owen, D.A.A., Wright, A., 1991. Preclinical pharmacology of ropinirole (SK&F 101468-A) a novel dopamine D2 agonist. Pharmacol. Biochem. Behav. 38, 147–154. https://doi.org/10.1016/0091-3057(91)90603-Y.

Ferraiolo, M., Atik, H., Ponthot, R., Belo do Nascimento, I., Beckers, P., Stove, C., Hermans, E., 2021. Receptor density influences ligand-induced dopamine D2L receptor homodimerization. Eur. J. Pharmacol. 911 https://doi.org/10.1016/j.ejphar.2021.174557.

Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

Frampton, J.E., 2019. Rotigotine transdermal patch: a review in Parkinson's disease. CNS Drugs 33, 707–718. https://doi.org/10.1007/s40263-019-00646-y.

Gay, E.A., Urban, J.D., Nichols, D.E., Oxford, G.S., Mailman, R.B., 2004. Functional selectivity of D2 receptor ligands in a Chinese hamster ovary hD2L cell line: evidence for induction of ligand-specific receptor states. Mol. Pharmacol. 66, 97–105. https://doi.org/10.1124/mol.66.1.97.

Gazi, L., Bobirnac, I., Danzeisen, M., Schüpbach, E., Langenegger, D., Sommer, B., Hoyer, D., Tricklebank, M., Schoeffter, P., 1999. Receptor density as a factor governing the efficacy of the dopamine D4 receptor ligands, L-745,870 and U-101958 at human recombinant D4.4 receptors expressed in CHO cells. Br. J. Pharmacol. 128, 613–620. https://doi.org/10.1038/sj.bjp.0702849.

Giladi, N., Boroojerdi, B., Korczyn, A.D., Burn, D.J., Clarke, C.E., Schapira, A.H.V., 2007. Rotigotine transdermal patch in early Parkinson's disease: a randomized, doubleblind, controlled study versus placebo and ropinirole. Mov. Disord. 22, 2398–2404. https://doi.org/10.1002/mds.21741.

Giladi, N., Nicholas, A.P., Asgharnejad, M., Dohin, E., Woltering, F., Bauer, L., Poewe, W., 2016. Efficacy of rotigotine at different stages of Parkinson's disease symptom severity and disability: a post hoc analysis according to baseline hoehn and yahr stage. J. Parkinsons Dis. 6, 741–749. https://doi.org/10.3233/JPD-160847.

Gray, R., Ives, N., Rick, C., Patel, S., Gray, A., Jenkinson, C., McIntosh, E., Wheatley, K., Williams, A., Clarke, C.E., Sandercock, P., Baigent, C., Crome, P., Abbott, R., Baker, M., Castleton, B., Counsell, C., Deb, A.K., Fairweather, S., Fitzpatrick, R., MacPhee, G., Malone, T., Mant, D., Ming, A., Morrish, P., Ohri, P., Pearce, V., Wood, B., Worth, P., Au, P., Boodell, T., Cheed, V., Daniels, J., Dowling, F., Edmondson, A., Hawker, R., Herd, C., Hilken, N., Kaur, S., Ottridge, R., Peto, L., Sidile, C., Tomlinson, C., Tyler, E., Winkles, N., Kent, S., Caie, L., Caslake, R., Coleman, R., Crowley, P., Gerrie, L., Gordon, J., Harris, C., Leslie, V., Macleod, M.A., Taylor, K., Barker, R., Forsyth, D., Halls, M., Lennox, G., Young, J., Azie, E., Barrett, J., Monaghan, A., Turnbull, C., Vanek, H., Blake, D., Manford, M., Thangarajah, N., Johnson, M., Wallis, P., Carr, P., Cochrane, L., Prescott, R., Rose, A., Drover, M., Karunaratne, P., McLaren, A., Jones, E., Nasar, M., Bayliss, M., Jones, A.P., Lewis, B., Dunn, A., Eckley, M., Price, J., Woodman, G., Aldridge, G., Bhuvanendran, N., Lewis, S., Mann, C., Patel, K., Ghaus, N., Grueger, A., Mallinson, B., Wihl, G., Ballantyne, S., Coyle, S., Hornabrook, R., Hutchinson, S., Irfan, H., Lewthwaite, A., Nicholl, D., Poxon, S., Ritch, A., Davison, J., Dodds, S., Gray, C., Nath, U., Robinson, G., Deb, A., Aftab, N., Read, D., Villanueva, L., Alderton, L., Burrows, E., Fletcher, P., Folkes, E., Gilbert, A., Hayes, H., Morrow, P., Silva, M., Baxter, G., Bell, J., Gorman, J., Lawrence, J., Rhind, G., Hindle, J., Jones, J., Parry, M., Roberts, E., Subashchandran, R., Pycock, C., Aspden, L., Partington-Jones, L., Raw, J., Wadhwa, U., Barber, R., Haywood, B., Heywood, P., Lewis, H., O'Sullivan, K., Prout, K., Whelan, L., Whone, A., Fuller, G., Medcalf, P., Aruldoss, P., Farmery, J., Liveley, K., Shelbourn, K., Sood, V., Bouifraden, K., Dalziel, J., Evans, C., Matheson, P., Overstall, P., Wales, E., Ward, G., Ponsford, J., Graham, I.A., Grimmer, S.F.M., Lockington, T.J., Sheehan, L.J., Williams, H., Fuller, J., Harrison, P., Roche, M., Shields, S., Glasspool, R., Hubbard, I., Walters, R., Barraclough, C., McClung, A., Moseley, L., Pathirana, C.K., Critchley, P., Wray, L.G., Kendall, B., Lawden, M., Lo, N., Martey, J., Rajabally, Y., Simpson, B., Gale, A., Phiri, D., Sekaran, L., Sharma, A., Wijayasiri, S., Fleary, H., Silverdale, M., Walker, D., McGee, M., Senthil, V., Reynolds, S., Arnould, D., Chong, S., Diem, D., Kundu, B., Quinn, N.,

Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

Benamer, H., Billings, J., Corston, R., D'Costa, D., Green, M., Shuri, J., Cassidy, T., Gani, A., Lawson, R., Noble, J., Chan, Y., Clipsham, K., Cochius, J., Dick, D., Hipperson, M., Lee, M., Payne, B., Reading, F., Sabanathan, K., Harper, G., Honan, W., Oxborough, L., Saunders, J., Stanley, J., McCann, P., Edmonds, P., Hand, A., O'Hanlon, S., Robinson, L., Walker, R., Bolam, D., Liddle, B., Ballantyne, S.L., Byravan, R., Jones, P., Guptha, S., Noble, C., Roychowdhury, S., Ellis, C., Harries -Jones, R., Hillier, C., Milligan, N., Potter, J., Ebenezer, L., Raha, S., Thompson, S., Crouch, R., Healy, K., Pall, H., Praamstra, P., Beaumont, D., Ell, S., McGourty, J., Jenkinson, M., McHenry, M., Scoble, N., Vahid, R., Findley, L., Misbahuddin, A., Adcock, J., Chatterjee, A., Collins, H., Flossman, E., Greenhall, R., Hart, H., Shaw, J., Singh, S., Talbot, K., Weir, A., Gray, D., Sutherland, S., Wilson, M., Hughes, T., Jones, A., Morgan, L., Sastry, B., Abdel-Hafiz, A., Al-Modaris, F., Dutta, S., Mallik, T., Mondal, B., Roberts, J., Sinha, S., Amar, K., Atkins, S., Devadason, G., Martin, A., Thompson, C., Fenwick, G., Gormley, K., Gutowski, N., Harris, S., Harrower, T., Hemsley, A., James, M., Jeffreys, O., Sheridan, R., Soper, C., Sword, J., Zeman, A., Gordon, C., McElwaine, T., Pressly, V., Chan, D., Saha, R., Howcroft, D., Mugweni, K., Robertson, D., Stephens, A., Whelan, E., Wright, A., Blane, N., Burns, S., Mutch, W., Roberts, R., Chikna, E., Chamberlain, J., Lee, J., Marigold, J., Adams, J., Dulay, J., Evans, S., Frankel, J., Garrard, P., Gibb, W., Gove, R., Holmes, C., Lawton, N., Malik, N., Morgan, S., Phipps, H., Queen, V., Roberts, H., Tan, R., Turner, G., Weller, R., Zaman, S., O'Brien, A., Grosset, D., McGonigal, A., Vennard, C., Rektorova, I., Carey, G., Dhakam, Z., Kalcantera, E., Long, C., Mandal, B., Martin, V., Nari, R., Nicholas, V., Sunderland, C., Franks, S., Hammans, S., Moffitt, V., Rice-Oxley, M., Elizabeth, J., Logan, A., Summers, B., Cooper, S., Darch, W., Homan, J., Hussain, M., Sharratt, D., Solanki, T., Bennett, J., Vassallo, J., Ford, A., Kendall, G., Stocker, K., Chaudhry, A., Grubneac, A., Kenton, A., Lindahl, A., Lismore, J., McConville, M., Peskett, R., Shehu, A., Strens, L., Moore, A., O'Brien, I., Watling, D., Wyatt, L., Jones, C., Mahan, T., Ullyart, K., Desai, H., Ferry, P., Ray, P., Rose, P., Thanvi, B., Waters, S., Rizvi, S., Sa'Adu, A., Walker, E., Berry, G., Russell, N., Ward, T., Abrams, J., Ashley, S., Steiger, M., Beal, A., Hawkins, J., Heller, A., Samuel, M., Primrose, W., Baker, K., Buckley, C., Bulley, S., Gibbons, D., Goodland, R., Jones, L., Martin, L., Qadiri, M.R., Rashed, K., Rowland -Axe, R., Stone, A., Whittuck, M., 2014. Long-term effectiveness of dopamine agonists and monoamine oxidase B inhibitors compared with levodopa as initial treatment for Parkinson's disease (PD MED): a large, open-label, pragmatic randomised trial. Lancet 384, 1196–1205. https://doi.org/10.1016/S0140-6736(14)60683-8.

Gundry, J., Glenn, R., Alagesan, P., Rajagopal, S., 2017. A practical guide to approaching biased agonism at G protein coupled receptors. Front. Neurosci. 11, 17. https://doi.org/10.3389/fnins.2017.00017.

Guo, W., Urizar, E., Kralikova, M., Mobarec, J.C., Shi, L., Filizola, M., Javitch, J.A., 2008. Dopamine D2 receptors form higher order oligomers at physiological expression levels. EMBO J. 27, 2293–2304. https://doi.org/10.1038/emboj.2008.153.

Han, Y., Moreira, I.S., Urizar, E., Weinstein, H., Javitch, J.A., 2009. Allosteric communication between protomers of dopamine class a GPCR dimers modulates activation. Nat. Chem. Biol. 5, 688–695. https://doi.org/10.1038/nchembio.199.

Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

Hermans, E., 2003. Biochemical and pharmacological control of the multiplicity of coupling at G-protein-coupled receptors. Pharmacol. Ther. 99, 25–44. https://doi.org/10.1016/S0163-7258(03)00051-2.

Hermans, E., Challiss, R.A.J., Nahorski, S.R., 1999. Effects of varying the expression level of recombinant human mGlu1α receptors on the pharmacological properties of agonists and antagonists. Br. J. Pharmacol. 126, 873–882. https://doi.org/10.1038/sj.bjp.0702359.

Ichise, M., Kim, Y.J., Ballinger, J.R., Vines, D., Erami, S.S., Tanaka, F., Lang, A.E., 1999. SPECT imaging of pre- and postsynaptic dopaminergic alterations in L- dopauntreated PD. Neurology 52, 1206–1214. https://doi.org/10.1212/wnl.52.6.1206.

Jarvis, G.E., Thompson, A.J., 2019. Evidence for an effect of receptor density on ligand occupancy and agonist EC 50. Sci. Rep. 9, 1–12. https://doi.org/10.1038/s41598-019-55361-x.

Kaasinen, V., Ruottinen, H.M., Någren, K., Lehikoinen, P., Oikonen, V., Rinne, J.O., 2000. Upregulation of putaminal dopamine D2 receptors in early Parkinson's disease: a comparative PET study with [11C]raclopride and [11C]Nmethylspiperone. J. Nucl. Med. 41, 65–70.

Kenakin, T., 2013. New concepts in pharmacological efficacy at 7TM receptors: IUPHAR Review 2. Br. J. Pharmacol. 168, 554–575. https://doi.org/10.1111/j.1476-5381.2012.02223.x.

Kenakin, T., 2009. A pharmacology primer. A Pharmacol. Prim 21–44. https://doi.org/10.1016/B978-0-12-374585-9.X0001-1.

Kenakin, T., 1997. Differences between natural and recombinant G protein-coupled receptor systems with varying receptor/G protein stoichiometry. Trends Pharmacol. Sci. 18, 456–464. https://doi.org/10.1016/S0165-6147(97)01136-X.

Kenakin, T., Watson, C., Muniz-Medina, V., Christopoulos, A., Novick, S., 2012. A simple method for quantifying functional selectivity and agonist bias. ACS Chem. Neurosci. 3, 193–203. https://doi.org/10.1021/cn200111m.

Kilts, J.D., Connery, H.S., Arrington, E.G., Lewis, M.M., Lawler, C.P., Oxford, G.S., O'Malley, K.L., Todd, R.D., Blake, B.L., Nichols, D.E., Mailman, R.B., 2002. Functional selectivity of dopamine receptor agonists. II. Actions of dihydrexidine in D2L receptor-transfected MN9D cells and pituitary lactotrophs. J. Pharmacol. Exp. Therapeut. 301, 1179–1189. https://doi.org/10.1124/jpet.301.3.1179.

Klewe, I.V., Nielsen, S.M., Tarpø, L., Urizar, E., Dipace, C., Javitch, J.A., Gether, U., Egebjerg, J., Christensen, K.V., 2008. Recruitment of β-arrestin2 to the dopamine D2 receptor: insights into anti-psychotic and anti-parkinsonian drug receptor signaling. Neuropharmacology 54, 1215–1222. https://doi.org/10.1016/j.neuropharm.2008.03.015.

Koener, B., Focant, M.C., Bosier, B., Maloteaux, J.M., Hermans, E., 2012. Increasing the density of the D 2L receptor and manipulating the receptor environment are required to evidence the partial agonist properties of aripiprazole. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 36, 60–70. https://doi.org/10.1016/j.pnpbp.2011.08.007.

Published in : Neuropharmacology (2022), vol. 207, 108942
DOI: 10.1016/j.neuropharm.2022.108942
Status : Postprint (Author's version)

Laschet, C., Dupuis, N., Hanson, J., 2019. A dynamic and screening-compatible nanoluciferase-based complementation assay enables profiling of individual GPCR–G protein interactions. J. Biol. Chem. 294, 4079–4090. https://doi.org/10.1074/jbc.RA118.006231.

Laschet, C., Hanson, J., 2021. Nanoluciferase-based complementation assay to detect GPCR-G protein interaction. Methods Mol. Biol. 2268, 149–157. https://doi.org/10.1007/978-1-0716-1221-7_10.

Lefkowitz, R.J., Shenoy, S.K., 2005. Transduction of receptor signals by β-arrestins. Science 308, 512–517. https://doi.org/10.1126/science.1109237.

Mann, A., Keen, A.C., Mark, H., Dasgupta, P., Javitch, J.A., Canals, M., Schulz, S., Robert Lane, J., 2021. New phosphosite-specific antibodies to unravel the role of GRK phosphorylation in dopamine D2 receptor regulation and signaling. Sci. Rep. 11 https://doi.org/10.1038/S41598-021-87417-2.

McCorvy, J.D., Butler, K.V., Kelly, B., Rechsteiner, K., Karpiak, J., Betz, R.M., Kormos, B. L., Shoichet, B.K., Dror, R.O., Jin, J., Roth, B.L., 2018. Structure-inspired design of β-arrestin-biased ligands for aminergic GPCRs. Nat. Chem. Biol. 14, 126–134. https://doi.org/10.1038/nchembio.2527.

Mierau, J., Schneider, F.J., Ensinger, H.A., Chio, C.L., Lajiness, M.E., Huff, R.M., 1995. Pramipexole binding and activation of cloned and expressed dopamine D2, D3 and D4 receptors. Eur. J. Pharmacol. Mol. Pharmacol. 290, 29–36. https://doi.org/10.1016/0922-4106(95)90013-6.

Mottola, D.M., Kilts, J.D., Lewis, M.M., Connory, H.S., Walker, Q.D., Jones, S.R., Booth, R.G., Hyslop, D.K., Piercey, M., Wightman, R.M., Lawler, C.P., Nichols, D.E., Mailman, R.B., 2002. Functional selectivity of dopamine receptor agonists. I. Selective activation of postsynaptic dopamine D 2 receptors linked to adenylate cyclase. J. Pharmacol. Exp. Therapeut. 301, 1166–1178. https://doi.org/10.1124/jpet.301.3.1166.

Nagi, K., Pineyro, G., 2016. Practical guide for calculating and representing biased signaling by GPCR ligands: a stepwise approach. Methods 92, 78–86. https://doi.org/10.1016/j.ymeth.2015.09.010.

Neve, K.A., Seamans, J.K., Trantham-Davidson, H., 2004. Dopamine receptor signaling. J. Recept. Signal Transduct. 24, 165–205. https://doi.org/10.1081/RRS-200029981.

Onaran, H.O., Ambrosio, C., Ugur, Ö., Madaras Koncz, E., Grò, M.C., Vezzi, V., Rajagopal, S., Costa, T., 2017. Systematic errors in detecting biased agonism: analysis of current methods and development of a new model-free approach. Sci. Rep. 7 https://doi.org/10.1038/srep44247.

Onaran, H.O., Costa, T., 2021. Conceptual and experimental issues in biased agonism. Cell. Signal. 82, 109955 https://doi.org/10.1016/j.cellsig.2021.109955.

Papenberg, G., Jonasson, L., Karalija, N., Johansson, J., Köhncke, Y., Salami, A., Andersson, M., Axelsson, J., Wåhlin, A., Riklund, K., Lindenberger, U., Lövdén, M., Nyberg, L., Bäckman, L., 2019. Mapping the landscape of human dopamine D2/3 receptors with [11C]raclopride. Brain Struct. Funct. 224, 2871–2882. https://doi.org/10.1007/s00429-019-01938-1.

Poewe, W.H., Rascol, O., Quinn, N., Tolosa, E., Oertel, W.H., Martignoni, E., Rupp, M., Boroojerdi, B., 2007. Efficacy of pramipexole and transdermal rotigotine in advanced Parkinson's disease: a double-

blind, double-dummy, randomized controlled trial. Lancet Neurol. 6, 513–520. https://doi.org/10.1016/S1474-4422(07)70108-4.

Politis, M., Wilson, H., Wu, K., Brooks, D.J., Piccini, P., 2017. Chronic exposure to dopamine agonists affects the integrity of striatal D2 receptors in Parkinson's patients. NeuroImage Clin 16, 455–460. https://doi.org/10.1016/j.nicl.2017.08.013.

Raeder, V., Boura, I., Leta, V., Jenner, P., Reichmann, H., Trenkwalder, C., Klingelhoefer, L., Chaudhuri, K.R., 2021. Rotigotine transdermal patch for motor and non-motor Parkinson's disease: a review of 12 Years' clinical experience. CNS Drugs 35, 215–231. https://doi.org/10.1007/s40263-020-00788-4.

Rinne, J.O., Laihinen, A., Ruottinen, H., Ruotsalainen, U., Någren, K., Lehikoinen, P., Oikonen, V., Rinne, U.K., 1995. Increased density of dopamine D2 receptors in the putamen, but not in the caudate nucleus in early Parkinson's disease: a PET study with [11C]raclopride. J. Neurol. Sci. 132, 156–161. https://doi.org/10.1016/0022-510X(95)00137-Q.

Rose, S.J., Pack, T.F., Peterson, S.M., Payne, K., Borrelli, E., Caron, M.G., 2018. Engineered D2R variants reveal the balanced and biased contributions of G-protein and β-arrestin to dopamine-dependent functions. Neuropsychopharmacology 43, 1164–1173. https://doi.org/10.1038/npp.2017.254.

Shen, Y., McCorvy, J.D., Martini, M.L., Rodriguiz, R.M., Pogorelov, V.M., Ward, K.M., Wetsel, W.C., Liu, J., Roth, B.L., Jin, J., 2019. D2 dopamine receptor G proteinbiased partial agonists based on cariprazine. J. Med. Chem. 62, 4755–4771. https://doi.org/10.1021/ACS.JMEDCHEM.9B00508/SUPPL_FILE/JM9B00508_SI_002.CSV.

Shonberg, J., Herenbrink, C.K., López, L., Christopoulos, A., Scammells, P.J., Capuano, B., Lane, J.R., 2013. A structure-activity analysis of biased agonism at the dopamine D2 receptor. J. Med. Chem. 56, 9199–9221. https://doi.org/10.1021/JM401318W/SUPPL_FILE/JM401318W_SI_001.PDF.

Singleton, S., Baptista-Hon, D.T., Edelsten, E., McCaughey, K.S., Camplisson, E., Hales, T. G., 2021. TRV130 partial agonism and capacity to induce anti-nociceptive tolerance revealed through reducing available μ-opioid receptor number. Br. J. Pharmacol. 178, 1855–1868. https://doi.org/10.1111/bph.15409.

Smith, J.S., Lefkowitz, R.J., Rajagopal, S., 2018. Biased signalling: from simple switches to allosteric microprocessors. Nat. Rev. Drug Discov. 17, 243–260. https://doi.org/10.1038/nrd.2017.229.

Stepniewski, T.M., Mancini, A., Ågren, R., Torrens-Fontanals, M., Semache, M., Bouvier, M., Sahlholm, K., Breton, B., Selent, J., 2021. Mechanistic insights into dopaminergic and serotonergic neurotransmission – concerted interactions with helices 5 and 6 drive the functional outcome. Chem. Sci. 12, 10990 https://doi.org/10.1039/D1SC00749A.

Urs, N.M., Bido, S., Peterson, S.M., Daigle, T.L., Bass, C.E., Gainetdinov, R.R., Bezard, E., Caron, M.G., 2015. Targeting β-arrestin2 in the treatment of L-DOPA-induced dyskinesia in Parkinson's disease. Proc. Natl. Acad. Sci. U.S.A. 112, E2517–E2526. https://doi.org/10.1073/pnas.1502740112.

Urs, N.M., Gee, S.M., Pack, T.F., McCorvy, J.D., Evron, T., Snyder, J.C., Yang, X., Rodriguiz, R.M., Borrelli, E., Wetsel, W.C., Jin, J., Roth, B.L., O'Donnell, P., Caron, M.G., Snyder, S.H., 2016. Distinct cortical and striatal actions of a β-arrestinbiased dopamine D2 receptor ligand reveal unique antipsychotic-like properties. Proc. Natl. Acad. Sci. U.S.A. 113, E8178–E8186. https://doi.org/10.1073/pnas.1614347113.

Verstappen, C.C.P., Bloem, B.R., Haaxma, C.A., Oyen, W.J.G., Horstink, M.W.I.M., 2007. Diagnostic value of asymmetric striatal D2 receptor upregulation in Parkinson's disease: an [123I]IBZM and [123I]FP-CIT SPECT study. Eur. J. Nucl. Med. Mol. Imag. 34, 502–507. https://doi.org/10.1007/s00259-006-0258-4.

Von Moo, E., Harpsøe, K., Hauser, A.S., Masuho, I., Bräuner-Osborne, H., Gloriam, D.E., Martemyanov, K.A., 2021. Ligand-directed bias of G protein signaling at the dopamine D2 receptor. Cell Chem. Biol. 0 (0) https://doi.org/10.1016/J.CHEMBIOL.2021.07.004.

Watts, V.J., Lawler, C.P., Gonzales, A.J., Zhou, Q.-Y., Civelli, O., Nichols, D.E., Mailman, R.B., 1995. Spare receptors and intrinsic activity: studies with D1 dopamine receptor agonists. Synapse 21, 177–187. https://doi.org/10.1002/syn.890210211.

Whalen, E.J., Rajagopal, S., Lefkowitz, R.J., 2011. Therapeutic potential of β-arrestinand G protein-biased agonists. Trends Mol. Med. 17, 126–139. https://doi.org/10.1016/j.molmed.2010.11.004.

Winkelmann, J., Allen, R.P., Högl, B., Inoue, Y., Oertel, W., Salminen, A.V., Winkelman, J.W., Trenkwalder, C., Sampaio, C., 2018. Treatment of restless legs syndrome: evidence-based review and implications for clinical practice (Revised 2017). Mov. Disord. 33, 1077–1091. https://doi.org/10.1002/mds.27260.

Wood, M., Dubois, V., Scheller, D., Gillard, M., 2015. Rotigotine is a potent agonist at dopamine D1 receptors as well as at dopamine D2 and D3 receptors. Br. J. Pharmacol. 172, 1124–1135. https://doi.org/10.1111/bph.12988.

Wouters, E., Marín, A.R., Dalton, J.A.R., Giraldo, J., Stove, C., 2019. Distinct dopamine D2 receptor antagonists differentially impact D2 receptor oligomerization. Int. J. Mol. Sci. 20 https://doi.org/10.3390/ijms20071686.

Zahoor, I., Shafi, A., Haq, E., 2018. Pharmacological treatment of Parkinson's disease. Park. Dis. Pathog. Clin. Asp. 129–144. https://doi.org/10.15586/codonpublications.parkinsonsdisease.2018.ch7.

Zhuo, C., Zhu, X., Jiang, R., Ji, F., Su, Z., Xue, R., Zhou, Y., 2017. Comparison for efficacy and tolerability among ten drugs for treatment of Parkinson's disease: a network meta-analysis. Sci. Rep. 8 https://doi.org/10.1038/srep45865.