Journal Pre-proof

Ondansetron, a highly selective 5-HT_3 receptor antagonist, reduces L-DOPA-induced dyskinesia in the 6-OHDA-lesioned rat model of Parkinson's disease

Cynthia Kwan, Imane Frouni, Dominique Bédard, Adjia Hamadjida, Philippe Huot

PII: S0014-2999(20)30006-6

DOI: https://doi.org/10.1016/j.ejphar.2020.172914

Reference: EJP 172914

To appear in: *European Journal of Pharmacology*

Received Date: 9 September 2019

Revised Date: 12 December 2019

Accepted Date: 7 January 2020

Please cite this article as: Kwan, C., Frouni, I., Bédard, D., Hamadjida, A., Huot, P., Ondansetron, a highly selective 5-HT_3 receptor antagonist, reduces L-DOPA-induced dyskinesia in the 6-OHDA-lesioned rat model of Parkinson's disease, *European Journal of Pharmacology* (2020), doi: https://doi.org/10.1016/j.ejphar.2020.172914.

Title: Ondansetron, a highly selective 5-HT_3 receptor antagonist, reduces L-DOPA-induced dyskinesia in the 6-OHDA-lesioned rat model of Parkinson's disease

Authors: Cynthia Kwan [1], Imane Frouni [1,2], Dominique Bédard [1], Adjia Hamadjida [1], Philippe Huot [1,2,3,4]

[1] Neurodegenerative Disease Group, Montreal Neurological Institute, Montreal, QC, Canada
[2] Département de Pharmacologie et Physiologie, Université de Montréal, Montreal, QC, Canada
[3] Department of Neurology and Neurosurgery, McGill University, Montreal, QC, Canada
[4] Department of Neurosciences, McGill University Health Centre, Montreal, QC, Canada

Text pages: 29
Word count abstract: 258
Word count introduction: 428
Word count discussion: 1518
Word count manuscript: 4326
Number of figures: 5
Number of tables: 0
Number of references: 75

Corresponding Author: Philippe Huot: Montreal Neurological Institute, 3801 University St, BTRC 205, Montreal, QC, Canada, H3A 2B4, Fax: +1-514-398-2304; Tel: +1-514-398-5957; Email: philippe.huot@mcgill.ca

Authors' roles
1) Research project: A. Conception, B. Organisation, C. Execution;
2) Manuscript: A. Writing of the first draft, B. Review and Critique.
Kwan: 1B, 1C, 2A, 2B; Frouni: 1C, 2B; Bédard: 1C, 2B; Hamadjida: 1B, 2A, 2B; Huot: 1A,1B,2B

Acknowledgements: PH has research support from Parkinson Canada, Fonds de Recherche Québec – Santé, the Weston Brain Institute, the Michael J Fox Foundation for Parkinson's Research, the Natural Sciences and Engineering Research Council of Canada and Healthy Brains for Healthy Lives.

Financial disclosure: There are no conflicts of interest. PH has received payments from Valeo Pharma.

Abstract

L-3,4-dihydroxyphenylalanine (L-DOPA) has been the standard treatment for Parkinson's disease (PD), despite that its chronic use leads to motor fluctuations and dyskinesia in as many as 95% of patients. Previous studies have shown that serotonin type 3 (5-HT_3) receptor blockade reduces dopamine levels within the striatum, suggesting that it could potentially lead to a reduction of dyskinesia. Here, we assessed the effects of ondansetron on L-DOPA-induced abnormal involuntary movements (AIMs) in the 6-hydroxydopamine (6-OHDA)-lesioned rat. We performed two series of experiments. First, rats exhibiting stable AIMs were administered ondansetron (0.0001, 0.001, 0.01, 0.1 and 1 mg/kg) or vehicle in combination with L-DOPA, following which the effect of ondansetron on AIMs was assessed. In the second series of experiments, following 6-OHDA lesion, rats received daily administration of ondansetron (0.0001, 0.001 mg/kg) or vehicle, started concurrently with the first L-DOPA dose, and treatments were continued for 22 days. After a washout period, an acute L-DOPA challenge was administered and AIMs severity was assessed. The effect of ondansetron on L-DOPA anti-parkinsonian action was also determined. We found that the addition of ondansetron 0.0001 mg/kg to L-DOPA resulted in a significant reduction of AIMs severity (by 31%, $P < 0.001$), when compared to vehicle. Ondansetron 0.0001 mg/kg, when started concurrently with L-DOPA, also attenuated the development of AIMs, with AIMs being 64% less severe ($P < 0.05$), when compared to L-DOPA/vehicle. Ondansetron did not impair L-DOPA anti-parkinsonian action. Our results suggest that selective 5-HT_3 blockade is a promising strategy to reduce the severity of L-DOPA-induced dyskinesia and may also attenuate its development.

Key words: ondansetron, Parkinson's disease, dyskinesia, 5-HT_3 receptor, 6-OHDA, rat

1. Introduction

Parkinson's disease (PD) is a neuro-degenerative disorder that affects approximately 1% of the population aged 60 years and over (de Lau and Breteler, 2006). Dopamine replacement therapy with L-3,4-dihydroxyphenylalanine (L-DOPA) is the most effective symptomatic treatment for PD, especially for bradykinesia and rigidity (Mercuri and Bernardi, 2005; Salat and Tolosa, 2013). However, with long-term L-DOPA intake, as many as 95% of PD patients develop complications, including motor fluctuations and involuntary movements, termed dyskinesia (Hely et al., 2005; Hely et al., 1999). Management of dyskinesia is limited to amantadine, a non-selective *N*-Methyl-D-aspartate (NMDA) antagonist with partial efficacy that is associated with adverse events (Hauser et al., 2017; Oertel et al., 2017; Pahwa and Hauser, 2017) or deep brain stimulation (Group, 2001; Rothlind et al., 2015; Williams et al., 2010) As such, there is an unmet therapeutic need to develop therapies for PD patients, as dyskinesia continues to undermine their quality of life (Thanvi et al., 2007).

A growing body of evidence suggests that the serotonin (5-HT) system plays a central role in the pathophysiology of dyskinesia (Huot and Fox, 2013), through the release of dopamine as a "false transmitter" (Carta et al., 2007). Agonists of 5-HT type 1A (5-HT_{1A}) and type 1B (5-HT_{1B}) auto-receptors suppressed the expression of L-DOPA-induced dyskinesia in the 6-hydroxydopamine (6-OHDA)-lesioned rat (Carta et al., 2007) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned non-human primate models (Bibbiani et al., 2001; Iravani et al., 2006). Moreover, administration of 5-HT type 2A (5-HT_{2A}) antagonists attenuated dyskinesia in the MPTP-lesioned marmoset (Hamadjida et al., 2018; Kwan et al., 2019b).

The 5-HT type 3 (5-HT_3) receptor is the sole ionotropic receptor of the 5-HT receptor family and its distribution in structures of the basal ganglia (Gehlert et al., 1991; Kilpatrick et al.,

1989; Laporte et al., 1992) suggests that it may modulate cerebral dopaminergic activity. Indeed, 5-HT_3 agonists stimulate striatal release of dopamine *in vitro* (Benuck and Reith, 1992; Blandina et al., 1988), an effect that is blocked by 5-HT_3 antagonists (Palfreyman et al., 1993).

Because of its effect on dopamine release, we hypothesised that antagonising 5-HT_3 receptors might translate to an anti-dyskinetic effect. Here, we have tested this hypothesis in the 6-OHDA-lesioned rat, utilising 2 experimental paradigms. We first assessed the effect of 5-HT_3 blockade on established dyskinesia, following which we evaluated the effect of antagonising 5-HT_3 receptors on the development of dyskinesia in the context of a *de novo* study. We have conducted our experiments with ondansetron, a potent and highly-selective 5-HT_3 receptor antagonist used in the clinic as an anti-emetic to control nausea and vomiting (Butcher, 1993; Marty et al., 1990).

2. Material and Methods

2.1 Animals

Adult female Sprague-Dawley rats (225 – 250 g, Charles River, Saint-Constant, Quebec, Canada) were housed in groups of 3 under conditions of controlled temperature (25 ± 1°C), humidity, light (12-h light/dark cycle, lights on at 07:00) with *ad libitum* access to food and water. Upon arrival, rats were left undisturbed to acclimatise to the housing conditions for at least 5 days before experiments. All experimental procedures described throughout this study were carried out in accordance with the guidelines of the Canadian Council on Animal Care

(CCAC), and approved by the Montreal Neurological Institute Animal Care Committee for protocol #2017-7922.

2.2 Drug treatments

Desipramine hydrochloride, pargyline hydrochloride, 6-OHDA hydrobromide, ascorbic acid, L-DOPA methyl ester hydrochloride, benserazide hydrochloride and ondansetron hydrochloride were purchased from MilliporeSigma, Canada. All drugs were dissolved in 0.9% NaCl unless otherwise specified. 6-OHDA hydrobromide was dissolved in 0.9% NaCl with 0.02% ascorbic acid, L-DOPA was dissolved in 0.9% NaCl with 0.1% ascorbic acid and ondansetron hydrochloride was dissolved in dimethyl sulfoxide at 100 mg/ml and then diluted to appropriate concentrations in 0.9% NaCl. All solutions were administered sub-cutaneously (s.c.) in a volume of 1 ml/kg body weight.

2.3 6-hydroxydopamine lesion

Rats were rendered hemi-parkinsonian as previously described (Frouni et al., 2019; Frouni et al., 2018; Huot et al., 2015). Briefly, rats were pre-treated with a solution of pargyline (5 mg/kg) and desipramine (10 mg/kg) to inhibit the uptake of 6-OHDA by noradrenergic neurons (Hamadjida et al., 2019). Thirty min later, rats were anaesthetised with isoflurane (2-4%; MilliporeSigma, Canada) in 100% oxygen (1 L/min) and positioned onto a stereotaxic frame (David Kopf Instruments, USA) with the incisor bar set 3.3 mm below ear bars. Rats were subsequently injected with 2.5 µL of 6-OHDA (7 µg/µl) in the right medial forebrain bundle at the following co-ordinates: antero-posterior : − 2.8 mm, medio-lateral: − 2.0 mm, dorso-ventral: − 9.0 mm) relative to bregma (Paxinos and Watson, 2018) using a 10-µl Hamilton Syringe

(MilliporeSigma, USA). 6-OHDA was injected at a flow rate of 0.5 µl/min and the syringe was left in place for an additional 5 min after injection before slow withdrawal of the needle to avoid reflux along the tract. At the end of the surgery, rats received s.c. injection of carprofen (10 mg/kg) and 0.9% NaCl (10 ml), to minimise post-surgical pain and avoid dehydration.

2.4 Experimental design

Experimental design for the acute challenges and *de novo* studies is described in Fig. 1.

2.4.1 Acute challenge study

Three weeks after lesion, following assessment of parkinsonism by the cylinder test (described in Section 2.5.1), rats (N = 18) exhibiting severe rearing asymmetry were selected to undergo daily priming with L-DOPA/benserazide (10/15 mg/kg) for 14 days to induce stable and reproducible axial, limbs and orolingual (ALO) abnormal involuntary movements (AIMs) (Frouni et al., 2018; Hamadjida et al., 2019). On days of behavioural testing, rats were administered L-DOPA (6/15 mg/kg, hereafter referred to as L-DOPA) in combination with ondansetron (0.0001, 0.001, 0.01 0.1 and 1 mg/kg) or vehicle, and the severity of ALO AIMs was assessed by a blinded rater. Treatments were randomised according to a within-subjects design and behavioural testing sessions were separated by at least 72 h. The doses of ondansetron were selected from previous pharmacokinetic (PK) experiments that we performed (Gaudette et al., 2019; Kwan et al., 2019b).

2.4.2 *De novo* study

In subsequent experiments, rats (N = 24) were rendered hemi-parkinsonian by 6-OHDA injection in the MFB as described above. Following a 21-day recovery period and assessment of the extent of lesion, rats were administered ondansetron 0.0001 mg/kg and 0.001 mg/kg (N = 9 for each group) or vehicle (N = 6), started concurrently with L-DOPA (6/15 mg/kg), once daily for 22 days. After a 3-day washout period, animals were administered an acute challenge of L-DOPA 6/15 mg/kg and ALO AIMs severity was assessed. The doses of ondansetron for the *de novo* experiments were the most effective ones identified during the acute challenge trials.

2.5 Behavioural assessment

2.5.1 Assessment of parkinsonism with the cylinder test

Following a 21-day post-lesion recovery period, for both acute challenge and *de novo* experiments, the degree of parkinsonism was assessed by the cylinder test (Schallert et al., 2000). Rats were placed in a transparent cylinder (15 cm diameter × 28 cm height) and a mirror was placed behind the cylinder to enable the evaluator to count forelimb movements when the animal was turned away from the camera. Animals were recorded for 10 min and the extent of forelimb use asymmetry displayed by the animal was analysed *post hoc*. The first limb to contact the wall during a rear or weight-shifting movement was scored as an independent wall placement for that limb. A subsequent placement of the other limb on the wall while maintaining the initial movement was scored as a "both" movement. A simultaneous placement of both forepaws on the walls was also considered a "both" movement. Another wall movement score was attributed only if both paws were removed from the vertical surface. Only animals exhibiting preferential use of

the un-lesioned forelimb in ≥ 70% of the rears were selected to undergo further behavioural pharmacological testing. This rearing asymmetry score is indicative of ≥ than 88% striatal dopamine depletion (Schallert et al., 2000).

2.5.2 Ratings of ALO AIMs

ALO AIMs were assessed by a proficient observer blinded to treatment, according to a scale previously described (Cenci and Lundblad, 2007; Dekundy et al., 2007; Lundblad et al., 2002). On days of behavioural scoring, following baseline assessment and treatment administration, rats were put in individual glass cylinders and ALO AIMs were rated for 2 min every 20 min over a 180 min testing session. ALO AIMs duration was rated on a scale from 0 to 4 in each monitoring interval, where: 0 = no dyskinesia; 1 = occasional signs of dyskinesia, present for less than 50% of the observation period; 2 = frequent signs of dyskinesia, present for more than 50% of the observation period; 3 = continuous dyskinesia but interrupted by external stimuli and 4 = continuous dyskinesia not interrupted by external stimuli. ALO AIMs amplitude was rated from 0 to 4. Axial AIMs consist of twisting of the neck and upper body toward the side contralateral to the lesion and their amplitude is rated according to the following scale: 1= sustained deviation of the head and neck at ~30° angle; 2 = sustained deviation of the head and neck at an angle of 60° or more; 3 = sustained twisting of the head, neck and upper trunk at an angle greater than 60° but up to 90° and 4 = sustained twisting of the head, neck and trunk at an angle greater than 90°, causing the rat to lose balance from a bipedal position. Limbs AIMs consist of jerky or dystonic movements of the contralateral limb and their amplitude is rated as follows: 1 = tiny movements of the paw around a fixed position; 2 = movements leading to visible displacement of the limb; 3 = large displacement of the limb with contraction of shoulder

muscles and 4 = vigorous limb displacement of maximal amplitude, with concomitant contraction of shoulder and extensor muscles. Orolingual AIMs consist of movement of jaw muscles and tongue protrusions and their amplitude is rated as: 1 = twitching of facial muscles accompanied by small masticatory movements without jaw opening; 2 = twitching of facial muscles accompanied by masticatory movements that occasionally result in jaw opening; 3 = movements with broad involvement of facial muscles and masticatory muscles, with frequent jaw opening and occasional tongue protrusion and 4 = involvement of all of the above muscles to the maximal possible degree. Cumulative ALO AIMs indicates the sum of ALO AIMs duration or of ALO AIMs amplitude over different consecutive measurement time points.

2.5.3 Assessment of L-DOPA anti-parkinsonian action

To determine whether any anti-dyskinetic effect of ondansetron would compromise the therapeutic efficacy of L-DOPA, rats used in the acute challenge study underwent a 3-day washout period, after which forelimb asymmetry was evaluated using cylinder test as described above. Rats were administered a low L-DOPA dose, 3/15 mg/kg, to avoid triggering AIMs, in combination with either ondansetron (0.0001, 0.001, 0.01, 0.1, 1 mg/kg) or vehicle, in a randomised within-subjects design, after which they underwent the cylinder test, 45 min later. Following the acute challenges study, animals were allowed a washout period prior to assessing L-DOPA anti-parkinsonian action with the cylinder test, to ensure that both drugs (L-DOPA and ondansetron) were completely eliminated from the body. L-DOPA and ondansetron have relatively short plasma half-lives, ≈ 59 min and ≈ 32 to 43 min, respectively, (Huot *et al.*, 2012; Kwan *et al.*, 2019a), and the 3-day washout period respects the recommended washout period of

a minimum of five times the half-life of the treatment with the longest half-life in the study (Evans, 2010).

2.6 Determination of striatal monoamine and metabolite levels

After the completion of behavioural experiments, rats were euthanised by isoflurane anaesthesia (2–4%; MilliporeSigma, Canada), followed by trans-cardial perfusion with 0.9% NaCl. Brains were rapidly removed, left and right striata were dissected on ice, flash frozen in 2-methyl-butane (-56°C) and stored at -80ºC until further analysis. High-performance liquid chromatography coupled with electro-chemical detection (HPLC-ED) was performed to determine striatal content of dopamine, 5-HT and their metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA), as previously described (Frouni et al., 2019; Frouni et al., 2018).

2.7 Statistical analysis

Data from the cylinder test to assess hemi-parkinsonism and the effects of ondansetron on L-DOPA anti-parkinsonian action are graphed as the mean ± standard error (S.E.M.) and were analysed using one-way repeated measures (RM) analysis of variance (ANOVA) followed by Tukey's *post hoc* tests. Cumulative AIMs scores in the acute challenge study are presented as the median with semi-interquartile range and were analysed using Friedman test followed by Dunn's *post hoc* test, while in the *de novo* study, cumulative AIMs scores are presented as the median with the semi-interquartile range and were analysed by Kruskal-Wallis followed by Dunn's *post hoc* tests. Tissue concentrations of monoamines and metabolites are presented as mean ± S.E.M. and were analysed by unpaired Welch's unequal variances *t* test. Statistical significance was set

to $P < 0.05$. Statistical analyses were performed with GraphPad Prism 8.0d (GraphPad Software Inc, USA).

3. Results

3.1 Extent of dopaminergic lesion

As shown in Fig. 2A, animals that underwent the acute challenges of ondansetron displayed forelimb asymmetry ($F_{(2, 39)} = 296.6$, $P < 0.001$, one-way RM ANOVA), with marked preferential use of the right forepaw in 83% of wall contacts when compared to 0.4% and 15% with the left forepaw and both forepaws, respectively (both $P < 0.001$, Tukey's *post hoc* test). These findings are consistent with the HPLC-ED analysis shown in Fig. 2B, where levels of dopamine and its metabolites DOPAC and HVA were significantly reduced in the lesioned striata compared to the un-lesioned striata [$t_{(14.04)} = 8.038$, $P < 0.001$ for dopamine], [$t_{(14.45)} = 8.011$, $P < 0.001$ for DOPAC] and [$t_{(15.20)} = 8.587$, $P < 0.001$ for HVA]. No difference was observed in the levels of 5-HT or 5-HIAA between striata of either hemisphere, [$t_{(27.84)} = 2.041$, $P > 0.05$ for 5-HT] and [$t_{(28.00)} = 0.9787$, $P > 0.05$ for 5-HIAA]. Striatal concentrations of dopamine, DOPAC and HVA diminished by 98%, 72% and 86%, respectively, (each $P < 0.001$) in the lesioned striata, compared to the un-lesioned striata.

3.2 Ondansetron diminishes the severity of established L-DOPA-induced ALO AIMs

As shown in Fig. 3A, administration of ondansetron in combination with L-DOPA reduced ALO AIMs duration (Friedman Statistic [FS] = 23.93, $P < 0.001$). Thus, administration

of ondansetron 0.0001 and 0.001 mg/kg decreased ALO AIMs duration by 31% and 29%, respectively, when compared to vehicle ($P < 0.001$ and $P < 0.01$, Dunn's *post hoc* test). ALO AIMs duration was also diminished with ondansetron 0.01 and 0.1 mg/kg compared to vehicle, but this was not statistically significant.

As shown in Fig. 3B, adding ondansetron to L-DOPA resulted in a significant reduction of ALO AIMs amplitude (FS = 30.07, $P < 0.001$). Thus, ondansetron 0.0001 and 0.001 mg/kg decreased ALO AIMs amplitude by 21% and 27%, respectively, when compared to vehicle ($P < 0.001$, Dunn's *post hoc* test).

3.3 Ondansetron does not impair the therapeutic efficacy of L-DOPA

Following acute challenges of ondansetron and a washout period, 6-OHDA-lesioned rats underwent a 3/15 mg/kg L-DOPA challenge to determine whether ondansetron treatment impairs L-DOPA anti-parkinsonian action, as measured by the cylinder test.

As presented in Fig. 4, administration of L-DOPA alone or in combination with ondansetron led to a decrease in the use of the right (un-lesioned) forepaw ($F_{(2.826, 39.56)} = 6.929$, $P < 0.001$, one-way RM ANOVA). When 6-OHDA-lesioned rats were administered L-DOPA, there was a significant decrease in the number of rears using the un-lesioned side (38%, $P < 0.05$, Tukey's *post hoc* test). This reduction in rears with the un-lesioned forepaw remained present when ondansetron 0.0001, 0.001, 0.01, 0.1 or 1 mg/kg was combined with L-DOPA, *i.e.* reductions of 37%, 48%, 44%, 58% and 47% were achieved, respectively (all $P < 0.01$ or $P < 0.001$, Tukey's *post hoc* test). There was no difference between the number of rears using the un-lesioned side between L-DOPA/vehicle and L-DOPA/ondansetron, regardless of the dose of ondansetron.

3.4 *De novo* treatment with ondansetron attenuates the development of ALO AIMs

After completion of the acute challenge experiments and having ensured that ondansetron does not interfere with L-DOPA anti-parkinsonian action, we sought to explore if ondansetron could attenuate the development of dyskinesia.

As shown in Fig. 5A, ALO AIMs duration was significantly milder in animals in which ondansetron was commenced concurrently with L-DOPA, compared to animals not exposed to ondansetron during the development phase (Kruskal-Wallis statistic [H] = 7.583, $P < 0.05$). However, despite statistical significance at the main test, no significant difference between treatment groups could be identified at the post test level ($P > 0.05$, Dunn's *post hoc* test), although rats administered ondansetron 0.0001 mg/kg during the priming phase exhibited ALO AIMs 43% less severe than animals treated with vehicle.

As depicted in Fig. 5B, the addition of ondansetron significantly attenuated the development of ALO AIMs amplitude ([H] = 8.064, $P < 0.05$). In animals primed with L-DOPA/ondansetron 0.0001 mg/kg, ALO AIMs amplitude was significantly lower by 64%, when compared to the L-DOPA/vehicle treatment ($P < 0.05$, Dunn's *post hoc* test).

4. Discussion

In the present study, we have demonstrated that selective blockade of the 5-HT_3 receptor with ondansetron reduces the severity of established L-DOPA-induced dyskinesia and attenuates the development of dyskinesia, in the 6-OHDA-lesioned rat, without interfering with the anti-parkinsonian action of L-DOPA.

Our experiments were performed solely on female rats, because their weight remains stable over time. Whereas, it remains uncertain if an anti-dyskinetic benefit would have been achieved in male rats, we believe it likely have resulted in a similar benefit in male animals. For instance, recent clinical trials performed with amantadine did not encounter different responses to glutamatergic dampening in male and female patients (Hauser *et al.*, 2017; Oertel *et al.*, 2017; Pahwa *et al.*, 2017)". In addition, in clinical settings, there are no differences between plasma levels achieved in male and female individuals following any given dose of L-DOPA (Dizdar *et al.*, 1999; Hauser *et al.*, 2011).

Our results are consistent with recent data obtained in the 6-OHDA-lesioned rat where concurrent administration of ondansetron with the initial dose of L-DOPA attenuated the development of dyskinesia (Aboulghasemi et al., 2018). In that recent study, after discontinuation of ondansetron treatment (0.04 and 0.08 mg/kg), animals exhibited a ≈ 27% and ≈ 54% reduction of dyskinesia compared to vehicle, which appears to be within the same magnitude as our findings (≈ 44%), despite that important methodological differences exist between the studies. Thus, in the Aboulghasemi et al. study, animals underwent the apomorphine rotation test to assess parkinsonism and therefore entered the *de novo* study without being completely drug naïve, which complicates the interpretation of the results, as apomorphine treatment may cause persistent behavioural effects (Silverman and Ho, 1981). Moreover, high doses of L-DOPA (50 mg/kg) were administered, without a peripheral L-amino acid decarboxylase inhibitor such as benserazide, which could perhaps explain why they obtained an anti-dyskinetic effect with relatively high doses of ondansetron (0.04 and 0.08 mg/kg), while we found the limit of efficacy with ondansetron 0.001 mg/kg when doses of L-DOPA/benserazide (6/15 mg/kg) commonly employed in the literature (Chambers et al., 2019; Lerner et al., 2017)

were administered. Nevertheless, the favourable data from both studies provide further support that 5-HT_3 receptor blockade might alleviate L-DOPA-induced dyskinesia.

In our study, ondansetron 0.0001 and 0.001 mg/kg consistently elicited the biggest AIMs reduction, while vehicle treatment led to the most severe AIMs, with the anti-dyskinetic effect disappearing when higher doses of ondansetron were administered. Such a bell shaped dose-response curve has often been ascribed to 5-HT_3 receptor antagonists (Goudie and Leathley, 1990; Ramamoorthy et al., 2008). The most favoured mechanism underlying this dose-response curve proposes that, at high concentrations of 5-HT_3 antagonists, there may be mutual steric hindrance at the receptor or, more speculatively, additional effects due to low-affinity binding to other receptors (Bonhaus et al., 1995; Eisensamer et al., 2003). Although ondansetron binds with low affinity to 5-HT_{1A}, 5-HT_{1B}, α-adrenergic and opioid receptors, its binding affinity at 5-HT_3 receptor sites is about 250- to 1000-fold higher than that at other receptors (van Wijngaarden et al., 1990). In addition, brain levels of ondansetron detected in a PK study suggest that the anti-dyskinetic action of ondansetron is possibly mediated exclusively by blockade of the 5-HT_3 receptor (Gaudette et al., 2019; Kwan et al., 2019a).

Here, the anti-dyskinetic efficacy obtained with ondansetron may therefore probably be attributed to its action as a 5-HT_3 receptor antagonist, especially considering its low affinity for other receptor subtypes. However, an important issue that remains to be addressed is whether the anti-dyskinetic effect and the bell-shaped dose-response curve represent a class effect, whereby drugs with a shared mechanism of action (*e.g.*, other 5-HT_3 receptor antagonists) would also alleviate L-DOPA-induced dyskinesia, but seemingly only at low dose, or if the effect would be limited to ondansetron. In addition to ondansetron, several highly-selective 5-HT_3 receptor antagonists such as granisetron, dolasetron and palonosetron are used in the clinic as anti-emetics

(Smith et al., 2012). Indeed, besides their shared mechanism of action, these compounds have different PK profiles and binding affinities (Hoyer, 1990), which may result in differences in their anti-dyskinetic potential. Further studies to investigate the possibility of a class effect by 5-HT_3 receptor antagonists in L-DOPA-induced dyskinesia are warranted.

L-DOPA-induced dyskinesia is thought to be mediated, in part, by the aberrant release of L-DOPA-derived dopamine from 5-HT neurons (Carta et al., 2007; Tanaka et al., 1999). Studies have demonstrated that 5-HT_3 receptors modulate the release of dopamine within the striatum, and dampening this release may be responsible for the anti-dyskinetic effect observed here. In rat striatal slices, administration of 5-HT_3 agonists increased endogenous levels of dopamine (Zazpe et al., 1994), while application of 5-HT_3 antagonists blocked dopamine release (Blandina et al., 1989). Pre-treatment with the 5-HT_3 antagonists ondansetron and MDL-72,222 also reduced the striatal increase of dopamine induced by dopaminergic drugs (Porras et al., 2003). An electrophysiological study suggested that the dampening of dopamine release in the striatum obtained with blockade of 5-HT_3 receptors might be attributed to the decrease of active dopaminergic neurons within the substantia nigra (Sorensen et al., 1989). Furthermore, in rodents, modulation of the 5-HT_3 receptor attenuated nigro-striatal dopamine transmission-mediated motor responses such as oro-facial dyskinesia (Naidu and Kulkarni, 2001), stereotypies (Shankar et al., 2000) and rotations (Bachy et al., 1993). In the mouse, unilateral injection of 5-HT_3 agonists in the striatum elicited contralateral rotations, which were suppressed by ondansetron (Bachy et al., 1993). Collectively, the studies cited above imply the involvement of the 5-HT_3 receptor in dopamine release in the striatum and it can thus be inferred that the anti-dyskinetic effect observed with ondansetron administration may, at least in part, be due to its inhibition on this excessive release of dopamine. However, this theory remains speculative

considering the lack of studies investigating the effect of ondansetron on striatal dopamine release in the 6-OHDA rat. Additional studies are required to corroborate whether this mechanism could be contributing to the anti-dyskinetic effect achieved with ondansetron, or whether, its action is mediated through different neurotransmitters or brain regions.

To the best of our knowledge, only one study has assessed the effects of ondansetron on physiological motor function in non-lesioned drug naïve rodents. Ondansetron treatment had no significant effects on locomotor activity in the rat, which is indicative of a lack of excitatory or suppressant effect (van der Hoek and Cooper, 1994). Experimental paradigms have studied drug-induced behaviours such as amphetamine-induced hyperactivity and cocaine-induced locomotion but as both drugs have the capacity to alter brain plasticity (Nyberg, 2014), extrapolating these findings to potential effects of ondansetron under drug naïve conditions is difficult. In the rat, following intra-nucleus accumbens (NAc) injection of amphetamine, central or peripheral administration of ondansetron did not significantly alter spontaneous locomotor activity (Costall et al., 1987). In contrast, ondansetron injection into the NAc attenuated hyperactivity induced by intra-NAc injection of amphetamine but failed to exert an effect when amphetamine was administered peripherally (Costall et al., 1987). Consistent with this finding, hyperlocomotion induced by intra-NAc infusion of dopamine was blocked by systemic administration of ondansetron in rats and marmosets (Costall et al., 1987), an effect that was also reported with other 5-HT_3 antagonists (Costall et al., 1990). Similarly, intra-NAc and intra-ventral tegmental area administration of ondansetron attenuated locomotor activity induced by peripheral administration of dexamphetamine (Gillies et al., 1996). Ondansetron also inhibited locomotor and head bobbing responses elicited by cocaine (Herges and Taylor, 2000). Collectively, these studies suggest that the mechanism underlying the ability of ondansetron to attenuate dopamine-

mediated hyperactivity is through reducing release of dopamine in the NAc (Herges and Taylor, 2000). To this end, an *in vivo* microdialysis study in the rat found that ondansetron significantly blocked the increase of dopamine in the accumbens elicited by stimulation of the dorsal raphe nucleus (De Deurwaerdere et al., 1998). Moreover, ondansetron treatment significantly reduced immobility time in the forced swim test and tail suspension test in mice (Kordjazy et al., 2016), which are validated behavioural tests for screening the anti-depressant effect of drugs (Cryan et al., 2002). The action of ondansetron was not due to changes in locomotion measured by the open field test (Kordjazy et al., 2016), which is consistent with the view that 5-HT_3 receptor plays a modulatory role only when the mesolimbic dopamine system is disturbed (Di Matteo et al., 2008).

Parkinsonism severity improved upon administration of an acute dose of L-DOPA and, importantly, this therapeutic benefit was maintained following administration of all doses of ondansetron in our experiments, which indicates that ondansetron does not impair the anti-parkinsonian action of L-DOPA. These findings are in agreement with a previous rat study (Aboulghasemi et al., 2018) and open-label trials in advanced PD patients in which ondansetron alleviated psychotic symptoms without compromising the therapeutic benefit conferred by L-DOPA (Friedberg et al., 1998; Zoldan et al., 1995).

In summary, we reported on the therapeutic potential of 5-HT_3 receptor blockade to diminish L-DOPA-induced dyskinesia. Ondansetron reduced dyskinesia without impairing the anti-parkinsonian action of L-DOPA, suggesting that it would be well tolerated in the clinic by PD patients with dyskinesia. Further studies are required to confirm these results and to uncover the mechanisms underlying the anti-dyskinetic efficacy of ondansetron.

References

Aboulghasemi, N., Hadipour Jahromy, M., Ghasemi, A., 2018. Anti-dyskinetic efficacy of 5-HT3 receptor antagonist in the hemi-parkinsonian rat model. IBRO Rep 6, 40-44.

Bachy, A., Héaulme, M., Giudice, A., Michaud, J.-C., Lefevre, I.A., Souilhac, J., Manara, L., Emerit, M.B., Gozlan, H., Hamon, M., Keane, P.E., Soubrié, P., Le Fur, G., 1993. SR 57227A: a potent and selective agonist at central and peripheral 5-HT3 receptors in vitro and in vivo. European journal of pharmacology 237, 299-309.

Benuck, M., Reith, M.E., 1992. Dopamine releasing effect of phenylbiguanide in rat striatal slices. Naunyn-Schmiedeberg's archives of pharmacology 345, 666-672.

Bibbiani, F., Oh, J.D., Chase, T.N., 2001. Serotonin 5-HT1A agonist improves motor complications in rodent and primate parkinsonian models. Neurology 57, 1829-1834.

Blandina, P., Goldfarb, J., Craddock-Royal, B., Green, J.P., 1989. Release of endogenous dopamine by stimulation of 5-hydroxytryptamine3 receptors in rat striatum. J Pharmacol Exp Ther 251, 803-809.

Blandina, P., Goldfarb, J., Green, J.P., 1988. Activation of a 5-HT3 receptor releases dopamine from rat striatal slice. Eur J Pharmacol 155, 349-350.

Bonhaus, D.W., Stefanich, E., Loury, D.N., Hsu, S.A.O., Eglen, R.M., Wong, E.H.F., 1995. Allosteric Interactions Among Agonists and Antagonists at 5-Hydroxytryptamine3 Receptors. J. Neurochem. 65, 104-110.

Butcher, M.E., 1993. Global experience with ondansetron and future potential. Oncology 50, 191-197.

Carta, M., Carlsson, T., Kirik, D., Bjorklund, A., 2007. Dopamine released from 5-HT terminals is the cause of L-DOPA-induced dyskinesia in parkinsonian rats. Brain 130, 1819-1833.

Cenci, M.A., Lundblad, M., 2007. Ratings of L-DOPA-induced dyskinesia in the unilateral 6-OHDA lesion model of Parkinson's disease in rats and mice. Curr Protoc Neurosci Chapter 9, Unit 9 25.

Chambers, N.E., Meadows, S.M., Taylor, A., Sheena, E., Lanza, K., Conti, M.M., Bishop, C., 2019. Effects of Muscarinic Acetylcholine m1 and m4 Receptor Blockade on Dyskinesia in the Hemi-Parkinsonian Rat. Neuroscience 409, 180-194.

Costall, B., Domeney, A.M., Naylor, R.J., 1990. 5-HT3 receptor antagonists attenuate dopamine-induced hyperactivity in the rat. Neuroreport 1, 77-80.

Costall, B., Domeney, A.M., Naylor, R.J., Tyers, M.B., 1987. Effects of the 5-HT3 receptor antagonist, GR38032F, on raised dopaminergic activity in the mesolimbic system of the rat and marmoset brain. Br J Pharmacol 92, 881-894.

Cryan, J.F., Markou, A., Lucki, I., 2002. Assessing antidepressant activity in rodents: recent developments and future needs. Trends in pharmacological sciences 23, 238-245.

De Deurwaerdere, P., Stinus, L., Spampinato, U., 1998. Opposite change of in vivo dopamine release in the rat nucleus accumbens and striatum that follows electrical stimulation of dorsal raphe nucleus: role of 5-HT3 receptors. J Neurosci 18, 6528-6538.

de Lau, L.M., Breteler, M.M., 2006. Epidemiology of Parkinson's disease. Lancet Neurol 5, 525-535.

Dekundy, A., Lundblad, M., Danysz, W., Cenci, M.A., 2007. Modulation of L-DOPA-induced abnormal involuntary movements by clinically tested compounds: further validation of the rat dyskinesia model. Behav Brain Res 179, 76-89.

Di Matteo, V., Pierucci, M., Esposito, E., Crescimanno, G., Benigno, A., Di Giovanni, G., 2008. Serotonin modulation of the basal ganglia circuitry: therapeutic implication for Parkinson's disease and other motor disorders. Prog Brain Res 172, 423-463.

Eisensamer, B., Rammes, G., Gimpl, G., Shapa, M., Ferrari, U., Hapfelmeier, G., Bondy, B., Parsons, C., Gilling, K., Zieglgänsberger, W., Holsboer, F., Rupprecht, R., 2003. Antidepressants are functional antagonists at the serotonin type 3 (5-HT3) receptor. Molecular Psychiatry 8, 994.

Friedberg, G., Zoldan, J., Weizman, A., Melamed, E., 1998. Parkinson Psychosis Rating Scale: a practical instrument for grading psychosis in Parkinson's disease. Clin Neuropharmacol 21, 280-284.

Frouni, I., Hamadjida, A., Kwan, C., Bedard, D., Nafade, V., Gaudette, F., Nuara, S.G., Gourdon, J.C., Beaudry, F., Huot, P., 2019. Activation of mGlu2/3 receptors, a novel therapeutic

approach to alleviate dyskinesia and psychosis in experimental parkinsonism. Neuropharmacology 158, 107725.

Frouni, I., Kwan, C., Bedard, D., Belliveau, S., Bourgeois-Cayer, E., Gaudette, F., Beaudry, F., Hamadjida, A., Huot, P., 2018. Effect of the selective 5-HT2A receptor antagonist EMD-281,014 on L-DOPA-induced abnormal involuntary movements in the 6-OHDA-lesioned rat. Exp Brain Res.

Gaudette, F., Bedard, D., Kwan, C., Frouni, I., Hamadjida, A., Beaudry, F., Huot, P., 2019. Highly sensitive HPLC-MS/MS assay for the quantitation of ondansetron in rat plasma and rat brain tissue homogenate following administration of a very low subcutaneous dose. J Pharm Biomed Anal 175, 112766.

Gehlert, D.R., Gackenheimer, S.L., Wong, D.T., Robertson, D.W., 1991. Localization of 5-HT3 receptors in the rat brain using [3H]LY278584. Brain Research 553, 149-154.

Gillies, D.M., Mylecharane, E.J., Jackson, D.M., 1996. Effects of 5-HT3 receptor-selective agents on locomotor activity in rats following injection into the nucleus accumbens and the ventral tegmental area. Eur J Pharmacol 303, 1-12.

Goudie, A.J., Leathley, M.J., 1990. Effects of the 5-HT3 antagonist GR38032F (ondansetron) on benzodiazepine withdrawal in rats. European journal of pharmacology 185, 179-186.

Group, T.D.-B.S.f.P.s.D.S., 2001. Deep-Brain Stimulation of the Subthalamic Nucleus or the Pars Interna of the Globus Pallidus in Parkinson's Disease. New England Journal of Medicine 345, 956-963.

Hamadjida, A., Frouni, I., Kwan, C., Huot, P., 2019. Classic animal models of Parkinson's disease: a historical perspective. Behav Pharmacol 30, 291-310.

Hamadjida, A., Nuara, S.G., Bedard, D., Gaudette, F., Beaudry, F., Gourdon, J.C., Huot, P., 2018. The highly selective 5-HT2A antagonist EMD-281,014 reduces dyskinesia and psychosis in the L-DOPA-treated parkinsonian marmoset. Neuropharmacology.

Hauser, R.A., Pahwa, R., Tanner, C.M., Oertel, W., Isaacson, S.H., Johnson, R., Felt, L., Stempien, M.J., 2017. ADS-5102 (Amantadine) Extended-Release Capsules for Levodopa-Induced Dyskinesia in Parkinson's Disease (EASE LID 2 Study): Interim Results of an Open-Label Safety Study. J Parkinsons Dis 7, 511-522.

Hely, M.A., Morris, J.G., Reid, W.G., Trafficante, R., 2005. Sydney Multicenter Study of Parkinson's disease: non-L-dopa-responsive problems dominate at 15 years. Mov Disord 20, 190-199.

Hely, M.A., Morris, J.G., Traficante, R., Reid, W.G., O'Sullivan, D.J., Williamson, P.M., 1999. The sydney multicentre study of Parkinson's disease: progression and mortality at 10 years. J Neurol Neurosurg Psychiatry 67, 300-307.

Herges, S., Taylor, D.A., 2000. Involvement of 5-HT(3) receptors in the nucleus accumbens in the potentiation of cocaine-induced behaviours in the rat. British journal of pharmacology 131, 1294-1302.

Hoyer, D., 1990. Serotonin 5-HT3, 5-HT4, and 5-HT-M receptors. Neuropsychopharmacology 3, 371-383.

Huot, P., Fox, S.H., 2013. The serotonergic system in motor and non-motor manifestations of Parkinson's disease. Exp Brain Res 230, 463-476.

Huot, P., Johnston, T.H., Koprich, J.B., Espinosa, M.C., Reyes, M.G., Fox, S.H., Brotchie, J.M., 2015. L-745,870 reduces the expression of abnormal involuntary movements in the 6-OHDA-lesioned rat. Behav Pharmacol 26, 101-108.

Iravani, M.M., Tayarani-Binazir, K., Chu, W.B., Jackson, M.J., Jenner, P., 2006. In 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated primates, the selective 5-hydroxytryptamine 1a agonist (R)-(+)-8-OHDPAT inhibits levodopa-induced dyskinesia but only with increased motor disability. J Pharmacol Exp Ther 319, 1225-1234.

Kilpatrick, G.J., Jones, B.J., Tyers, M.B., 1989. Binding of the 5-HT3 ligand, [3H]GR65630, to rat area postrema, vagus nerve and the brains of several species. European journal of pharmacology 159, 157-164.

Kordjazy, N., Haj-Mirzaian, A., Amiri, S., Ostadhadi, S., Amini-khoei, H., Dehpour, A.R., 2016. Involvement of N-methyl-d-aspartate receptors in the antidepressant-like effect of 5-

hydroxytryptamine 3 antagonists in mouse forced swimming test and tail suspension test. Pharmacology Biochemistry and Behavior 141, 1-9.

Kwan, C., Bedard, D., Frouni, I., Gaudette, F., Beaudry, F., Hamadjida, A., Huot, P., 2019a. Plasma and brain pharmacokinetics of the selective 5-HT3 receptor antagonist ondansetron in the rat Journal of Pharmacology and Experimental Therapeutics.

Kwan, C., Frouni, I., Bedard, D., Nuara, S.G., Gourdon, J.C., Hamadjida, A., Huot, P., 2019b. 5-HT2A blockade for dyskinesia and psychosis in Parkinson's disease: is there a limit to the efficacy of this approach? A study in the MPTP-lesioned marmoset and a literature mini-review. Exp Brain Res 237, 435-442.

Laporte, A.M., Koscielniak, T., Ponchant, M., Vergé, D., Hamon, M., Gozlan, H., 1992. Quantitative autoradiographic mapping of 5-HT3 receptors in the rat CNS using [125I]iodo-zacopride and [3H]zacopride as radioligands. Synapse (New York, N.Y.) 10, 271-281.

Lerner, R.P., Francardo, V., Fujita, K., Bimpisidis, Z., Jourdain, V.A., Tang, C.C., Dewey, S.L., Chaly, T., Cenci, M.A., Eidelberg, D., 2017. Levodopa-induced abnormal involuntary movements correlate with altered permeability of the blood-brain-barrier in the basal ganglia. Sci Rep 7, 16005.

Lundblad, M., Andersson, M., Winkler, C., Kirik, D., Wierup, N., Cenci, M.A., 2002. Pharmacological validation of behavioural measures of akinesia and dyskinesia in a rat model of Parkinson's disease. Eur J Neurosci 15, 120-132.

Marty , M., Pouillart , P., Scholl , S., Droz , J.P., Azab , M., Brion , N., Pujade-Lauraine , E., Paule , B., Paes , D., Bons , J., 1990. Comparison of the 5-Hydroxytryptamine3 (Serotonin) Antagonist Ondansetron (Gr 38032F) with High-Dose Metoclopramide in the Control of Cisplatin-Induced Emesis. New England Journal of Medicine 322, 816-821.

Mercuri, N.B., Bernardi, G., 2005. The 'magic' of L-dopa: why is it the gold standard Parkinson's disease therapy? Trends in pharmacological sciences 26, 341-344.

Naidu, P.S., Kulkarni, S.K., 2001. Reversal of neuroleptic-induced orofacial dyskinesia by 5-HT3 receptor antagonists. European journal of pharmacology 420, 113-117.

Nyberg, F., 2014. Structural plasticity of the brain to psychostimulant use. Neuropharmacology 87, 115-124.

Oertel, W., Eggert, K., Pahwa, R., Tanner, C.M., Hauser, R.A., Trenkwalder, C., Ehret, R., Azulay, J.P., Isaacson, S., Felt, L., Stempien, M.J., 2017. Randomized, placebo-controlled trial of ADS-5102 (amantadine) extended-release capsules for levodopa-induced dyskinesia in Parkinson's disease (EASE LID 3). Mov Disord 32, 1701-1709.

Pahwa, R., Hauser, R.A., 2017. ADS-5102 (Amantadine) Extended Release for Levodopa-Induced Dyskinesia. JAMA Neurol 74, 1507-1508.

Pahwa, R., Tanner, C.M., Hauser, R.A., Isaacson, S.H., Nausieda, P.A., Truong, D.D., Agarwal, P., Hull, K.L., Lyons, K.E., Johnson, R., Stempien, M.J., 2017. ADS-5102 (Amantadine)

Extended-Release Capsules for Levodopa-Induced Dyskinesia in Parkinson Disease (EASE LID Study): A Randomized Clinical Trial. JAMA Neurol 74, 941-949.

Palfreyman, M.G., Schmidt, C.J., Sorensen, S.M., Dudley, M.W., Kehne, J.H., Moser, P., Gittos, M.W., Carr, A.A., 1993. Electrophysiological, biochemical and behavioral evidence for 5-HT2 and 5-HT3 mediated control of dopaminergic function. Psychopharmacology (Berl) 112, S60-67.

Paxinos, G., Watson, C., 2018. The rat brain in stereotaxic coordinates, Compact seventh edition. ed. Elsevier Science, San Diego.

Porras, G., De Deurwaerdere, P., Moison, D., Spampinato, U., 2003. Conditional involvement of striatal serotonin3 receptors in the control of in vivo dopamine outflow in the rat striatum. Eur J Neurosci 17, 771-781.

Ramamoorthy, R., Radhakrishnan, M., Borah, M., 2008. Antidepressant-like effects of serotonin type-3 antagonist, ondansetron: an investigation in behaviour-based rodent models. Behav Pharmacol 19, 29-40.

Rothlind, J.C., York, M.K., Carlson, K., Luo, P., Marks, W.J., Weaver, F.M., Stern, M., Follett, K., Reda, D., 2015. Neuropsychological changes following deep brain stimulation surgery for Parkinson's disease: comparisons of treatment at pallidal and subthalamic targets versus best medical therapy. Journal of Neurology, Neurosurgery & Psychiatry 86, 622-629.

Salat, D., Tolosa, E., 2013. Levodopa in the treatment of Parkinson's disease: current status and new developments. J Parkinsons Dis 3, 255-269.

Schallert, T., Fleming, S.M., Leasure, J.L., Tillerson, J.L., Bland, S.T., 2000. CNS plasticity and assessment of forelimb sensorimotor outcome in unilateral rat models of stroke, cortical ablation, parkinsonism and spinal cord injury. Neuropharmacology 39, 777-787.

Shankar, R.P., Karan, R.S., Handu, S.S., Bhargava, V.K., 2000. Effect of the 5-HT3 receptor antagonist ondansetron on amphetamine-induced hyperactivity and stereotypy in rats. Indian journal of physiology and pharmacology 44, 355-358.

Silverman, P.B., Ho, B.T., 1981. Persistent behavioural effect in apomorphine in 6-hydroxydopamine-lesioned rats. Nature 294, 475-477.

Smith, H.S., Cox, L.R., Smith, E.J., 2012. 5-HT3 receptor antagonists for the treatment of nausea/vomiting. Ann Palliat Med 1, 115-120.

Sorensen, S.M., Humphreys, T.M., Palfreyman, M.G., 1989. Effect of acute and chronic MDL 73,147EF, a 5-HT3 receptor antagonist, on A9 and A10 dopamine neurons. European journal of pharmacology 163, 115-118.

Tanaka, H., Kannari, K., Maeda, T., Tomiyama, M., Suda, T., Matsunaga, M., 1999. Role of serotonergic neurons in L-DOPA-derived extracellular dopamine in the striatum of 6-OHDA-lesioned rats. Neuroreport 10, 631-634.

Thanvi, B., Lo, N., Robinson, T., 2007. Levodopa-induced dyskinesia in Parkinson's disease: clinical features, pathogenesis, prevention and treatment. Postgrad Med J 83, 384-388.

van der Hoek, G.A., Cooper, S.J., 1994. Ondansetron, a selective 5-HT3 receptor antagonist, reduces palatable food consumption in the nondeprived rat. Neuropharmacology 33, 805-811.

van Wijngaarden, I., Tulp, M.T., Soudijn, W., 1990. The concept of selectivity in 5-HT receptor research. European journal of pharmacology 188, 301-312.

Williams, A., Gill, S., Varma, T., Jenkinson, C., Quinn, N., Mitchell, R., Scott, R., Ives, N., Rick, C., Daniels, J., Patel, S., Wheatley, K., 2010. Deep brain stimulation plus best medical therapy versus best medical therapy alone for advanced Parkinson's disease (PD SURG trial): a randomised, open-label trial. The Lancet Neurology 9, 581-591.

Zazpe, A., Artaiz, I., Del Río, J., 1994. Role of 5-HT3 receptors in basal and K(+)-evoked dopamine release from rat olfactory tubercle and striatal slices. Br. J. Pharmacol. 113, 968-972.

Zoldan, J., Friedberg, G., Livneh, M., Melamed, E., 1995. Psychosis in advanced Parkinson's disease: treatment with ondansetron, a 5-HT3 receptor antagonist. Neurology 45, 1305-1308.

Figure legends

Fig. 1. Schematic representation of the experimental design.

(A) Timeline of the acute challenge experiments. 6-OHDA lesioned animals underwent a L-DOPA priming phase to induce dyskinesia and the effect of acute ondansetron on the severity of AIMs was evaluated.

(B) Timeline of the *de novo* experiments. Animals were concomitantly administered ondansetron with L-DOPA daily over the course of 21 days with weekly assessments of the progression of AIMs, followed by an acute L-DOPA challenge after a washout period.

Fig. 2. Extent of striatal dopaminergic denervation.

(A) Animals selected to undergo acute challenges of ondansetron exhibited a marked preference for the un-lesioned (right) forepaw in 83% of rears compared to 0.4% and 17% of rears using the lesioned (left) forepaw and both forepaws, respectively. Data are presented as the mean ± S.E.M. ***: $P < 0.001$.

(B) HPLC-ED analysis revealed that striatal levels of dopamine and metabolites 3,4-dihydroxyphenylacetic acid and homovanillic acid were significantly reduced in the right striatum when compared to the left striatum (by 98%, 72% and 86% respectively). Striatal levels of serotonin and 5-hydroxyindoleacetic acid were similar in both striata. Data are presented as the mean ± S.E.M. ***: $P < 0.001$.

Fig. 3. Effect of acute challenges of ondansetron on established L-DOPA induced AIMs

(A) Administration of ondansetron 0.0001 and 0.001 mg/kg in combination with L-DOPA attenuated axial limbs orolingual AIMs duration, by 31% and 29%, respectively, when compared to vehicle.

(B) Treatment with ondansetron 0.0001 and 0.001 mg/kg resulted in a marked reduction in the amplitude of axial limbs orolingual AIMs, by 21% and 27%, when compared to vehicle.

Data are expressed as median with semi-interquartile interval. **: $P < 0.01$, ***: $P < 0.001$.

Fig. 4. Effect of ondansetron on L-DOPA anti-parkinsonian action.

Drug-naïve 6-OHDA-lesioned rats used the right (un-lesioned) forepaw in 83% of rears. When 6-OHDA-lesioned rats were administered L-DOPA (3/15 mg/kg), there was a significant decrease in the number of rears using the un-lesioned side, by 39%. This decrease in rears with the un-lesioned forepaw remained present when ondansetron 0.0001, 0.001, 0.01, 0.1 or 1 mg/kg was combined with L-DOPA by 37%, 47%, 44%, 58% and 49%, respectively. Data are graphed as mean ± S.E.M. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

Fig. 5. Effect of ondansetron on axial limbs orolingual AIMs during an acute 6/15 mg/kg L-DOPA challenge following chronic administration of ondansetron during the AIMs induction phase.

(A) Rats that received L-DOPA/ondansetron 0.0001 mg/kg during the induction phase did not exhibit significantly milder axial limbs orolingual duration, when compared to animals treated chronically with L-DOPA/vehicle.

(B) Administration of L-DOPA/ondansetron 0.0001 mg/kg during the induction phase significantly attenuated the development of axial limbs orolingual AIMs amplitude compared to animals treated with L-DOPA/vehicle (by 64%, $P < 0.05$).

Data are graphed as median with semi-interquartile range. *: $P < 0.05$

Fig. 1

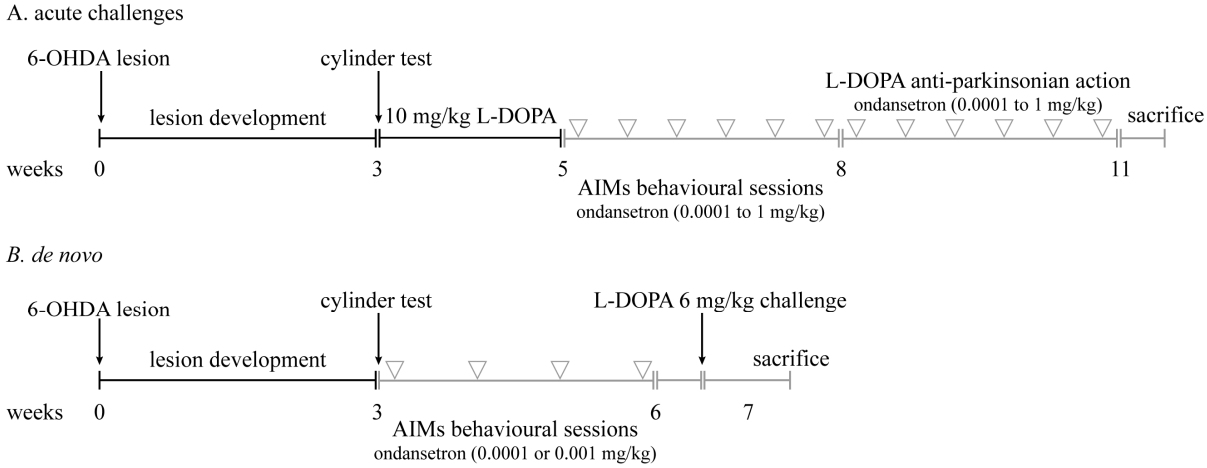

Fig. 2

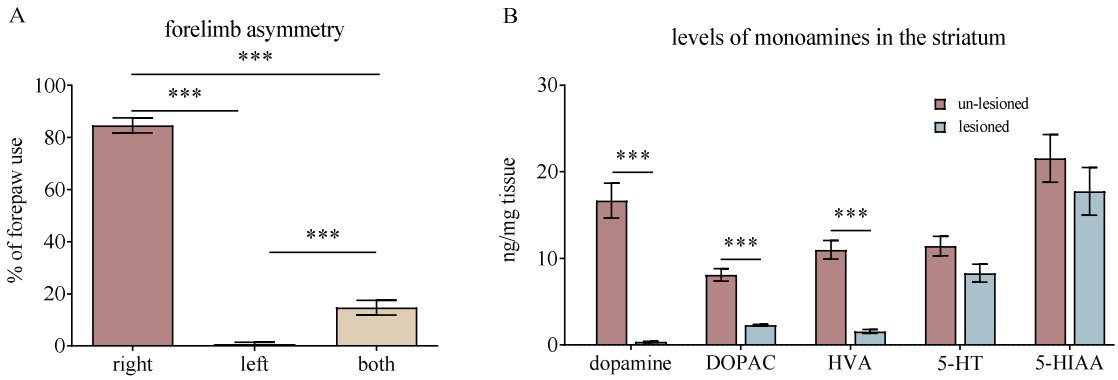

Fig. 3

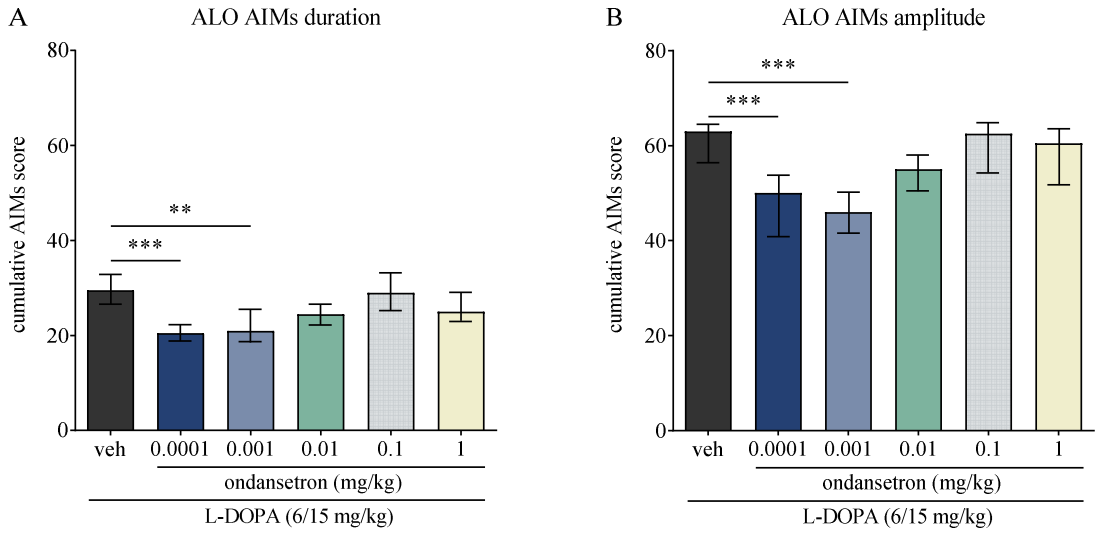

Fig. 4

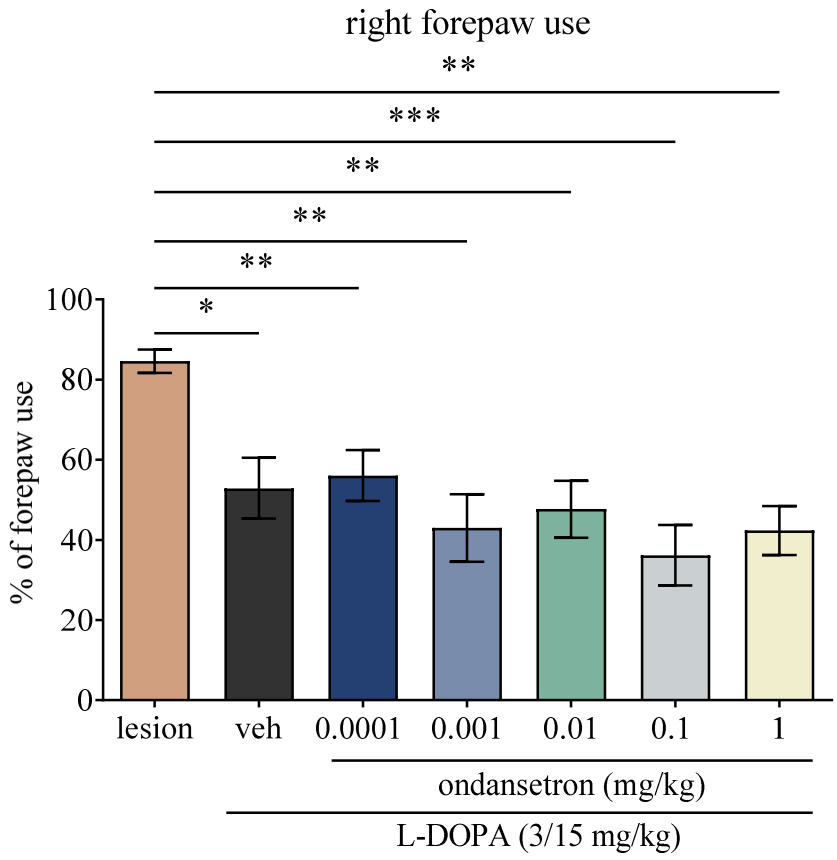

Fig. 5