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UWA-121, a mixed dopamine and serotonin re-uptake inhibitor, enhances L-DOPA anti-parkinsonian action without worsening dyskinesia or psychosis-like behaviours in the MPTP-lesioned common marmoset

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ABSTRACT

L-3,4-Dihydroxyphenylalanine (L-DOPA) is the most effective treatment for Parkinson's disease (PD), but its long-term administration is complicated by wearing-off and dyskinesia. UWA-101, a dual, equipotent inhibitor of dopamine (DAT) and serotonin (SERT) transporters, has previously been shown to successfully extend duration of anti-parkinsonian benefit of L-DOPA (ON-time), without exacerbating dyskinesia, in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned marmoset. However, UWA-101 is racemic and it is unclear whether one or both enantiomers contribute to its actions, and whether a better therapeutic effect might be attained by using a single antipode. In the current study, we synthesised the two enantiomers of UWA-101, R-101 (UWA-121) and S-101 (UWA-122), characterised their pharmacological profiles and administered them to MPTP-lesioned marmosets. Parkinsonism, dyskinesia, psychosis-like behaviours and duration of ON-time were evaluated. UWA-121 is a dual DAT > SERT inhibitor, with an approximate 10:1 DAT:SERT affinity ratio (inhibitory constants (Ki) of 307 and 3830 nM, respectively). In combination with L-DOPA, UWA-121 extended duration of ON-time when compared to L-DOPA/vehicle treatment (by 40%, $P < 0.01$). UWA-121 also extended duration of ON-time without dyskinesia (by 215%, $P < 0.05$) and ON-time without psychosis-like behaviours when compared to L-DOPA/vehicle treatment (by 345%, $P < 0.01$). UWA-121 did not worsen the severity of dyskinesia or psychosis-like behaviours ($P > 0.05$). UWA-122 is a selective SERT inhibitor (Ki 120 nM, Ki at DAT > 50 μM) and, in combination with L-DOPA, had no effect on ON-time, dyskinesia or psychosis-like behaviours ($P > 0.05$). These data indicate that dual DAT and SERT inhibitors effectively enhance L-DOPA anti-parkinsonian action without worsening dyskinesia and that compounds with such a pharmacological profile represent promising agents against wearing-off in PD.

1. Introduction

Parkinson's disease (PD) is characterised by the degeneration of dopaminergic neurons of the substantia nigra, leading to a deficiency of striatal dopamine (Hornykiewicz and Kish, 1987; Marsden, 1982). As such, the dopamine precursor L-3,4-dihydroxyphenylalanine (L-DOPA) is the mainstay of PD treatment (Fahn, 2008). However, long-term L-DOPA administration is marred by the emergence of motor complications, *e.g.* dyskinesia and loss of benefit (wearing-off), and non-motor complications, *e.g.* psychosis. After 15 years of therapy, motor complications affect 95% of patients, whereas 50% of patients experience non-motor symptoms (Hely et al., 2005).

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An effective treatment for wearing-off, *i.e.* one that does not exacerbate dopaminergic side effects such as dyskinesia and psychosis, remains elusive. Thus, although current therapies against wearing-off, such as the monoamine oxidase (MAO) B inhibitor rasagiline, and the catechol-O-methyltransferase (COMT) inhibitor entacapone, effectively extend duration of ON-time, they do it at the expense of worsening dyskinesia (Penney et al., 2002; Parkinson Study Group, 1997, 2005; Rascol et al., 2005; Rinne et al., 1998; Talati et al., 2009).

Inhibition of dopamine re-uptake from the synaptic cleft should enhance dopaminergic transmission and is therefore a potentially useful approach against wearing-off. Monoamine re-uptake inhibitors have long been studied in PD (Matthews, 1938; Solomon et al., 1937), but the quest for an efficacious drug remains ongoing. For example, when administered with L-DOPA to PD patients, the dual dopamine transporter (DAT) and noradrenaline transporter (NET) inhibitor nomifensine had a favourable effect on parkinsonism, but worsened dyskinesia (Teychenne et al., 1976), and failed to enhance L-DOPA anti-parkinsonian action in another study (Bedard et al., 1977). Tesofensine, a triple inhibitor with relatively similar potency at the DAT, NET and serotonin (5-HT) transporter (SERT), produced neither a sustained anti-parkinsonian benefit as monotherapy in recently diagnosed PD patients (Hauser et al., 2007), nor an enhancement of L-DOPA anti-parkinsonian benefit as adjunct therapy (Bara-Jimenez et al., 2004). Moreover, in a Phase II trial, while tesofensine reduced daily OFF-time, it exacerbated dyskinesia (Rascol et al., 2008).

The failure of the monoamine re-uptake inhibitors to provide clinical benefit against wearing-off might be because an effective profile of monoamine transporter inhibition has not yet been defined. Our recent discovery of the 3,4-methylenedioxymethamphetamine (MDMA) analogue, UWA-101 (Fig. 1), a dual inhibitor with approximately equal affinity for DAT and SERT, led us to propose that dual DAT/SERT inhibition might represent a better balance with respect to extending ON-time without exacerbating dyskinesia. Although the experience with UWA-101 is currently limited to pre-clinical settings, the drug significantly extends ON-time duration, without exacerbating dyskinesia, in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned marmoset (Huot et al., 2012a; Johnston et al., 2012). However, UWA-101 appears to exacerbate L-DOPA-induced psychosis-like behaviours at higher doses (Huot et al., 2012a). Given the known pathophysiology of dyskinesia and psychosis, we proposed that the relative DAT:SERT inhibition ratio of UWA-101 may not be optimal for therapeutic use.

UWA-101 is a racemate and, in the current study, in order to determine its mechanism of action and define how its beneficial effects might be taken forward towards clinical development, we have synthesised the two enantiomers of UWA-101, R-101 (UWA-121), and S-101 (UWA-122), characterised their pharmacological profiles and behavioural actions, in combination with L-DOPA, in the MPTP-lesioned marmoset. The MPTP-lesioned marmoset has been extensively validated to study both motor and non-motor complications, including dyskinesia and psychosis-like behaviours, as well as to assess duration of ON-time (Fox et al., 2010, 2006b; Gomez-Ramirez et al., 2006; Huot et al., 2012a, 2011; Jenner et al., 1984; Johnston et al., 2011, 2012; Pearce et al., 1995; Visanji et al., 2008, 2006).

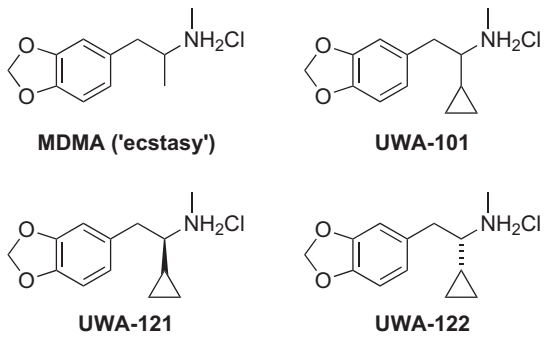


Fig. 1. Monoamine re-uptake inhibitors relevant to this study.

2. Materials and methods

2.1. Synthesis of UWA-121 and -122

The enantiomers UWA-121 and UWA-122 were obtained by a classical resolution of molecular diastereomers, using methodology inspired by Shulgin and co-workers' first synthesis of enantiopure R- and S-MDMA (Nichols et al., 1986). Reductive amination of piperonyl cyclopropyl ketone (**1**) (Gandy et al., 2010) with R-α-methylbenzylamine (**2**), gave a mixture of diastereomers (**3**) (Fig. 2). *N*-Methylation allowed flash chromatographic separation of the resultant diastereomeric tertiary amines (**4**). Separate, hydrogenolytic removal of the chiral auxiliary and treatment with methanolic HCl gave the hydrochlorides UWA-121 and UWA-122, the configuration and enantiopurity of which were established by X-ray crystallography.

2.2. In vitro pharmacology

2.2.1. Tissue preparation

Female Sprague–Dawley rats (250–275 g, Charles River, Saint-Constant, Canada) were housed in a temperature (19–21 °C), humidity (55%) and light-controlled (12 h light/dark cycle, lights on 07:00) environment with unlimited access to food and water. All procedures were approved by University Health Network Animal Care Committee and conducted in accordance with the regulations defined by the Canadian Council on Animal Care. All reasonable efforts were made to reduce animal numbers used to the minimum required to employ rigorous statistical analyses and minimise their suffering.

Rats were sacrificed by decapitation following CO_2 narcosis. Brains were immediately removed and placed into ice-cold Krebs buffer (134 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 1 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.25 mM KH_2PO_4, and 10 mM glucose). Various brain regions (cerebral cortex, striatum, cerebellum and remaining brain) were dissected on ice and placed separately into ice-cold Tris buffer (pH 7.4) prior to probe sonication on ice. Brain homogenates were then centrifuged (20,000 × g_{max}) for 20 min at 4 °C, re-suspended into ice-cold Tris buffer (pH 7.4), and re-centrifuged for 20 min at 4 °C (20,000 × g_{max}). Supernatant was then removed, the pellet was re-suspended in ice-cold Tris buffer (pH 7.4), vortexed and placed in a 37 °C water bath for 20 min, to metabolise endogenous neurotransmitters. Following a final centrifugation step (20 min at 4 °C, 20,000 × g_{max}), supernatant was removed and the pellet was re-suspended in ice-cold Tris buffer (pH 7.4). Protein concentration was determined by Lowry variant of the Folin's phenol reagent method (Lowry et al., 1951).

2.2.2. Radioligands and drugs

[^{3}H]-Ketanserin (to label serotonergic type 2A [5-HT_{2A}] receptors, specific activity: 67 Ci/mmol), [^{3}H]-GBR-12,935 (to label the DAT, specific activity: 43 Ci/mmol), [^{3}H]-nisoxetine (to label the NET, specific activity: 85 Ci/mmol), [^{3}H]-citalopram (to label the SERT, specific activity: 84 Ci/mmol), [^{3}H]-dopamine (specific activity: 38.7 Ci/mmol), [^{3}H]-noradrenaline (specific activity: 14.8 Ci/mmol) and [^{3}H]-5-HT (specific activity: 27.7 Ci/mmol) were purchased from PerkinElmer (Waltham, USA). Spiperone, maprotiline, paroxetine, GBR-12,909 and OR-486 were purchased from Tocris Bioscience (Ellisville, USA). Nialamide, dopamine, noradrenaline and 5-HT were purchased from Sigma–Aldrich (St Louis, USA).

2.2.3. Receptor and transporter binding assays

The assays described here were used to define the pharmacological profiles of UWA-121/122. The receptor and transporter binding assays performed in the current study targeted only the receptor/transporters to which UWA-101 was shown to exhibit affinity in previous experiments performed by our group (Johnston et al., 2012), *i.e.* 5-HT_{2A} receptors and each of the DAT, SERT and NET. Brain synaptosomes were incubated with UWA-121/122 (1 nM–1 μM) in the presence of the radioligand in Tris (pH 7.4) buffer. Incubations were conducted in 380 μL 96-well plates. Binding parameters relating to brain region used, ligand and ion concentrations, as well as incubation conditions, are provided in Table 1.

After incubation, the membranes were rapidly washed using a cell harvester (Brandel, Gaithersburg, USA) in 50 mM Tris buffer (pH 7.4; 20 s wash at 10 mL/s) and filtered under vacuum through Whatman glass fibre (G/F) filters (GE Healthcare Canada, Mississauga, Canada) pre-soaked in 50 mM Tris buffer (pH 7.4). For NET and DAT assays, G/F filters were pre-soaked in a 50 mM Tris (pH 7.4) solution containing 0.1% polyethyleneimine (PEI; Sigma–Aldrich, St Louis, USA) and were washed with a

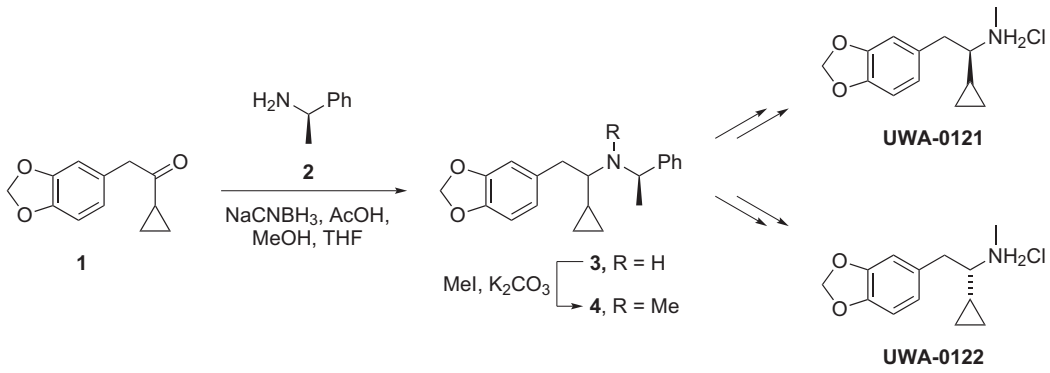


Fig. 2. Synthesis of enantiopure UWA-121 and UWA-122.

50 mM Tris solution (pH 7.4; 20 s wash at 10 mL/s) containing 0.1% bovine serum albumin (BSA; Sigma–Aldrich, St Louis, USA). Filters were then immersed in scintillation fluid (Ecoscint, Atlanta, USA). Radioactivity was determined with a scintillation counter (Beckman Coulter, Mississauga, Canada) as counts per minute.

2.2.4. Monoamine re-uptake assays

The assays described here were performed to determine the functional effect of UWA-121/122 on the three monoaminergic transporters. Monoamine re-uptake assays were performed for each of the DAT, NET and SERT as described before (Johnston et al., 2012). Re-uptake assays were performed in Krebs buffer (134 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 1 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.25 mM KH_2PO_4, 10 mM glucose, 100 μM ascorbic acid, pH 7.4) in the presence of OR-486 (COMT inhibitor, for dopamine and noradrenaline re-uptake assays only) and nialamide (MAO inhibitor). Brain synaptosomes were pre-incubated with UWA-121/122 (1 nM–1 μM) at 37 °C for 10 min, after which radiolabeled and unlabelled monoamine were added. Incubations were conducted in 380 μL 96-well plates. Experimental parameters relating to brain region used, radiolabeled and unlabelled monoamine concentrations, ion concentration, as well as incubation conditions are displayed in Table 2.

After incubation, the membranes were rapidly washed using a cell harvester in a similar way as described above for the binding experiments. For the dopamine and 5-HT assays, G/F filters were pre-soaked in Tris buffer (pH 7.4) containing 0.1% PEI, whereas G/F filters were pre-soaked in Tris buffer (pH 7.4) containing 0.1% BSA for the noradrenaline assay. The washing solution was Tris buffer (pH 7.4) for the dopamine and 5-HT assays, whereas it was 135 mM NaCl for the noradrenaline assay.

2.2.5. Determination of UWA-121/122 affinity at selected receptor/transporters

Values of displacement by UWA-121/122 were determined in three independent experiments, each in triplicate, and displacement expressed as a percentage of specific binding. Dose–response curves were constructed and the half-maximal inhibitory concentration (IC_{50}) was determined via non-linear regression analysis using GraphPad Prism 5.03 (GraphPad Software Inc, La Jolla, USA) software. The inhibition constant (Ki) was calculated using the Cheng–Prusoff equation (Cheng and Prusoff, 1973). The binding affinity and monoamine re-uptake potency of UWA-121 and UWA-122 are presented as the mean ± standard error (SEM) Ki.

2.3. Behavioural assessment of UWA-121/122 in the MPTP-lesioned non-human primate

2.3.1. Induction of parkinsonism and dyskinesia in the common marmoset

Six female common marmosets (*Callithrix jacchus*; Harlan, Madison, USA), weighing 350–500 g, were housed in groups of 2–3 under conditions of controlled temperature (25 ± 2 °C), humidity (55%) and a 12 h light/dark cycle (08:00 lights on). Animals were cared for in accordance with a protocol approved by University Health Network Animal Care Committee in accordance with the regulations defined by the Canadian Council on Animal Care. Access to food, fresh fruit supplements and water was unrestricted. Home cages were enriched with primate toys, perches and auditory stimuli. Prior to the start of studies, animals were acclimatised to handling, administration of subcutaneous (s.c.) treatments, as well as transfer to observation cages for behavioural assessment.

Parkinsonism was induced by injections of MPTP hydrochloride (2 mg/kg s.c. daily, for 5 consecutive days) (Sigma–Aldrich, St Louis, USA). The MPTP administration phase was followed by a 12 week recovery period to allow parkinsonian symptoms to develop and stabilise. Treatment-related complications, including dyskinesia and psychosis-like behaviours, were elicited by treatment with oral Prolopa® (L-DOPA/benserazide 15/3.75 mg/kg twice daily; Hoffmann-La Roche Limited, Mississauga, Canada) for a minimum of 30 days. This treatment regimen has been previously demonstrated to produce a stable model of L-DOPA-induced motor and non-motor complications (Fox et al., 2010; Gomez-Ramirez et al., 2006).

2.3.2. Administration of UWA-121/122, in combination with L-DOPA, to parkinsonian marmosets

On days of behavioural assessment, at 09:00, marmosets were injected with a therapeutic dose of L-DOPA/benserazide 15/3.75 mg/kg s.c. (Sigma–Aldrich, St Louis, USA) in combination with either vehicle (NaCl 0.9%) or UWA-121/122 hydrochloride, (equivalent to 1, 3 and 10 mg/kg of drug free base s.c.). Drug administration schedule was randomised, both within and between animals, according to a Latin square design (Experimental Design Generator And Randomiser [EDGAR] www.edgarweb.org.uk/). After administration of a given treatment, each marmoset was placed individually into an observation cage (0.8 × 0.8 × 0.7 m) containing food, water and a wooden perch, and left undisturbed for the 6 h duration of the experiment. Behaviour was recorded on DVD and analysed *post hoc* by a movement disorders neurologist blinded to the treatment given. At least 48 h were left between each treatment in any animal.

2.3.3. Motor activity

A quantitative assessment of marmoset activity was made using computer-operated passive infrared sensors as described previously (Johnston et al., 2011). A single sensor containing a hemispherical lens (Guardall, Mississauga, Canada) was mounted 1.5 m over the top of each observation cage. The sensor position allowed motion to be detected throughout the entire cage below. The signal was fed via an RS-232 input to a computer. Proprietary Motion Detector software (Research Electronics, Toronto, Canada) was used that displayed activity counts within Microsoft Office Excel (Microsoft, Redmond, USA). Total activity counts were logged in 30 min epochs across the 6 h duration of the experiment.

Table 1
Experimental conditions for receptor/transporters binding assays.

Receptor/transporter	Brain tissue	Radioligand (concentration)	Non-specific displacer	Ionic conditions	Incubation parameters
$5HT_{2A}$	Cerebral cortex	[^{3}H]-ketanserin (2.5 nM)	Spiperone	–	45 min, 4 °C
DAT	Striatum	[^{3}H]-GBR-12,935 (5.6 nM)	GBR-12,909	125 mM NaCl	45 min, room T°
NET	Cerebellum	[^{3}H]-nisoxetine (2.0 nM)	Maprotiline	300 mM NaCl, 5 mM KCl	60 min, 4 °C
SERT	ROB	[^{3}H]-citalopram (2.5 nM)	Paroxetine	120 mM NaCl, 5 mM KCl	60 min, room T°

$5HT_{2A}$: serotonergic type 2A receptors; DAT: dopamine transporter; NET: norepinephrine transporter; SERT: serotonin transporter.
ROB: remainder of brain, corresponding to the brain areas remaining following dissection of the cortex, striatum, and cerebellum. ROB contains the raphe nuclei, substantia nigra, thalamus, globus pallidus, hippocampus, amygdala, and subcortical white matter. Brain tissue concentration remained fixed for all of the experiments (2 mg/mL of protein). The non-specific displacer concentration remained unchanged for all of the experiments (10 μM).

Table 2
Experimental conditions for monoamine re-uptake assays.

Transporter	Brain tissue	Radioligand (concentration)	Final monoamine concentration	Non-specific displacer	Incubation parameters
DAT	Striatum	[^{3}H]-dopamine (10 nM)	225 nM	GBR-12,909	10 min, 37 °C
NET	Cerebellum	[^{3}H]-noradrenaline (10 nM)	1 μM	Maprotiline	5 min, 37 °C
SERT	ROB	[^{3}H]-5-HT (10 nM)	150 nM	Paroxetine	5 min, 37 °C

5-HT: serotonin; DAT: dopamine transporter; NET: noradrenaline transporter; SERT: serotonin transporter.
ROB: remainder of brain, corresponding to the brain areas remaining following dissection of the cortex, striatum, and cerebellum. ROB contains the raphe nuclei, substantia nigra, thalamus, globus pallidus, hippocampus, amygdala, and subcortical white matter. Brain tissue concentration remained fixed for all of the experiments (2 mg/mL of protein). The non-specific displacer concentration remained unchanged for all of the experiments (10 μM).

2.3.4. Behavioural analysis

The scales used for assessment of behaviour were described in detail previously (Fox et al., 2010, 2006b; Gomez-Ramirez et al., 2006; Huot et al., 2011; Visanji et al., 2006). Parkinsonian disability was rated for 5 min every 10 min using a parkinsonian disability scale combining measures of range of movement, bradykinesia, posture, and attention/alertness. Range of movement was rated on a 0–9 scale: 0 = running, jumping between roof, walls, perch, using limbs through a wide range of activity; 1 = climbing up and down the walls of the cage or along perch; 2 = climbing onto wall of cage or perch; 3 = hopping on floor of cage; 4 = walking around floor; 5 = on wall of cage or perch, movement of limbs, but no locomotion; 6 = on wall of cage or perch, movement of head or trunk; 7 = floor of the cage, movement of limb, but no locomotion; 8 = on the floor of the cage, movement of head; 9 = no movement. Bradykinesia was rated from 0 to 3: 0 = normal initiation and speed of movement; 1 = slight slowing of movement; 2 = moderate slowing of movement, marked freezing, difficulty initiating and maintaining movement; 3 = prolonged freezing, akinesia, inability to move. Postural abnormalities were rated 0 or 1: 0 = normal balance, upright posture, head held up; 1 = impaired balance, crouched posture, head down. Attention/alertness was rated 0 or 1; 0 = normal head checking movements, movement of neck in variable directions, smooth, small movements; 1 = reduced or absent head checking, head in one position for more than 50% of observation period. The score attributed to each of the behaviours assessed was the most representative of the 5 min observation period. A global parkinsonian disability score was calculated as a combination of the behaviours mentioned above, equally weighted, according to the following formula: (range of movement × 1) + (bradykinesia × 3) + (posture × 9) + (alertness × 9). The maximal parkinsonian disability score per 5 min observation period was 36.

L-DOPA-induced dyskinesia and psychosis-like behaviours were also assessed. Both dyskinesia and psychosis-like behaviours were rated from 0 to 4. For dyskinesia, 0 = absent; 1 = mild, fleeting, present less than 30% of the observation period; 2 = moderate, not interfering with normal activity, present more than 30% of the observation period; 3 = marked, at times interfering with normal activity, present less than 70% of the observation period; 4 = severe, continuous, replacing normal activity, present more than 70% of the observation period. In any 5 min period of assessment, choreiform and dystonic dyskinesia were graded separately and the dyskinesia score given reflected the most disabling dyskinesis observed, whether chorea or dystonia. The following psychosis-like behaviours were assessed: hyperkinesia, response to non-apparent stimuli (hallucinatory behaviour), repetitive grooming and stereotypies. Each of these was rated from 0 to 4, where 0 = absent; 1 = present for <30% of assessment time and not disabling (animal can walk, run, and eat); 2 = present for > 30% of assessment time and not disabling; 3 = present for < 30% and disabling (interferes with walking, running, eating − takes over normal activity); 4 = present for > 30% and disabling. For any 5 min period of assessment, the psychosis-like behaviours score attributed was the most disabling of any of the four sub-scores observed. Several articles assessing psychosis-like behaviours in the MPTP-lesioned marmoset have been published (Fox et al., 2010, 2006b; Huot et al., 2012a; Huot et al., 2011; Visanji et al., 2006) and the scale herein used to rate behaviours has been detailed and validated in (Fox et al., 2010).

Parkinsonian disability, dyskinesia and psychosis-like behaviours scores were cumulated for each hour across the entire 6 h of observation and during the peak effect period (90–150 min following L-DOPA administration). The duration of anti-parkinsonian benefit, ON-time, was defined as the number of minutes for which bradykinesia was absent (score 0). ON-time was further divided as "good" or "bad" quality, depending on the severity of dyskinesia present. "Good quality" ON-time was defined as the number of minutes when bradykinesia was 0 and dyskinesia were either absent or non-disabling, *i.e.* mild, or moderate in intensity (scores of 0, 1 or 2), whereas "bad quality" ON-time was defined as the number of minutes during which bradykinesia was 0 and dyskinesia were disabling, *i.e.* either marked or severe (scores of 3 or 4). ON-time without disabling dyskinesia was defined as the sum of ON-time without dyskinesia and ON-time with non-disabling dyskinesia (scores of 0, 1 or 2). Similarly, psychosis-like behaviours scores of 0, 1 or 2 were considered non-disabling, whereas scores of 3 or 4 were considered disabling.

2.4. Statistical analysis

Continuous motor activity scores are presented as the mean or the mean ± SEM and were analysed by one-way repeated measures analysis of variance (RM ANOVA) followed by Dunnett's *post hoc* tests (except time course data, see below). Categorical, discontinuous scores for parkinsonian disability, dyskinesia and psychosis-like behaviours severity are presented as the median or the median with individual values and were analysed using non-parametric Friedman followed by Dunn's *post hoc* tests (except time course data, see below). ON-time data are presented as the mean ± SEM and were analysed by one-way RM ANOVA followed by Tukey's *post hoc* tests. Time course data for motor activity counts were analysed by a two-way RM ANOVA followed by Bonferroni's *post hoc* tests. Time course data for parkinsonian disability, dyskinesia and psychosis-like behaviours scores were ranked by marmoset across each of the four treatments and analysed by a two-way ANOVA followed by Bonferroni's *post hoc* tests. Statistical significance was assigned when $P < 0.05$. Statistical analyses were computed using GraphPad Prism 5.03 (GraphPad Software Inc, La Jolla, USA).

3. Results

3.1. Pharmacological profiles of UWA-121/122

UWA-121 exhibited high affinity for DAT (Ki: 307 ± 33 nM) and moderate affinity for SERT (Ki: 3830 ± 760 nM). In contrast, UWA-122 exhibited no affinity for DAT (Ki > 50 μM), but displayed high affinity for SERT (Ki: 120 ± 31 nM) (Table 3). Neither UWA-121 nor UWA-122 bound to NET (Ki > 50 μM for both).

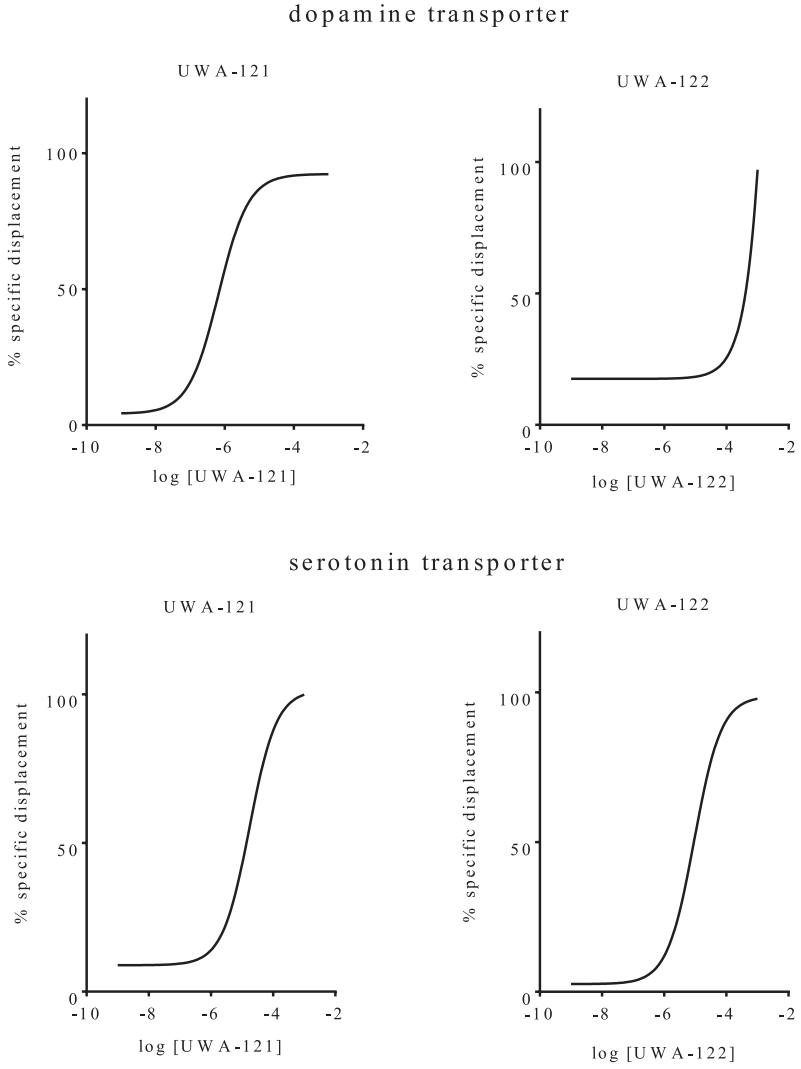
UWA-121 potently inhibited the re-uptake of dopamine (Ki: 592 ± 130 nM) and moderately inhibited the re-uptake of 5-HT (Ki: 4640 ± 880 nM) in functional assays. UWA-121 did not inhibit the re-uptake of noradrenaline (Ki > 50 μM). UWA-122 potently inhibited the re-uptake of 5-HT (Ki: 340 ± 67 nM), but did not inhibit the re-uptake of dopamine or noradrenaline (Ki > 50 μM for both) (Table 4).

3.2. UWA-121, but not UWA-122, increases motor activity induced by L-DOPA

Time course analysis revealed that UWA-121 (10 mg/kg), in combination with L-DOPA, significantly increased motor activity when compared to L-DOPA/vehicle treatment in the time periods 60–120 min and 120–180 min post treatment ($F_{time}(5, 90) = 20.30$, $P < 0.001$, $F_{treatment}(3, 90) = 8.172$, $P < 0.001$, and $F_{interaction}(15, 90) = 1.493$, $P > 0.05$, two-way RM ANOVA, $P < 0.05$ from 60 to 120 min and $P < 0.001$ from 120 to 180 min, Bonferroni's *post hoc* test; Fig. 3A). L-DOPA/UWA-121 (10 mg/kg) also increased motor activity from 120 to 180 min when compared to L-DOPA/UWA-121 (1 and 3 mg/kg) ($P < 0.01$ and $P < 0.05$ respectively, Bonferroni's *post hoc* test). In contrast, UWA-122 (1, 3 and 10 mg/kg) did not increase L-DOPA-induced motor activity when compared to vehicle treatment, but UWA-122 (1 mg/kg) decreased L-DOPA-induced motor activity throughout the observation period (Fig. 3C); this decrease in motor activity was significant from 120 to 180 min when L-DOPA/UWA-122 1 mg/kg was compared to L-DOPA/UWA-122 10 mg/kg, but not when compared to L-DOPA/vehicle ($F_{time}(5, 90) = 23.93$, $P < 0.001$, $F_{treatment}(3, 90) = 3.185$, $P < 0.05$, and $F_{interaction}(15, 90) = 0.5133$, $P > 0.05$, two-way RM ANOVA, $P < 0.05$ Bonferroni's *post hoc* test).

UWA-121 also increased total L-DOPA-induced motor activity cumulated over the whole observation period. Thus, mean total motor activity counts were 5541 ± 444 in the L-DOPA/vehicle

Table 3
UWA-121/122 binding profiles with dose–response curves at the dopamine and serotonin transporters.



	5-HT_{2A}	NET	SERT	DAT
UWA-121	>50,000	>50,000	3830 ± 760	307 ± 33
UWA-122	>50,000	>50,000	120 ± 31	>50,000

The affinity is provided as the mean Ki (nM) ± SEM of three independent experiments, each performed in triplicate.
5-HT_{2A}: serotonergic type 2A receptors; DAT: dopamine transporter; NET: noradrenaline transporter; SERT: serotonin transporter.

group, whereas they were 7226 ± 937 following treatment with L-DOPA/UWA-121 10 mg/kg (a 31% increase, $F(5, 15) = 3.646$, $P < 0.05$, one-way RM ANOVA, $P < 0.05$, Dunnett's *post hoc* test, Fig. 3B). Combining UWA-122 (1, 3 and 10 mg/kg) and L-DOPA did not change total motor activity from 0 to 360 min when compared to L-DOPA/vehicle treatment ($F(5, 15) = 1.604$, $P > 0.05$, one-way RM ANOVA, Fig. 3D).

3.3. UWA-121 extends duration of L-DOPA anti-parkinsonian action, and provides "good quality" extra ON-time

UWA-121 (10 mg/kg) significantly extended duration of L-DOPA anti-parkinsonian action when compared to L-DOPA/vehicle and L-DOPA in combination with lower doses of UWA-121 (1 and 3 mg/kg) ($F(5, 15) = 6.605$, $P < 0.01$, one-way RM ANOVA, Fig. 4A). Thus, duration of ON-time was 268 ± 24 min after L-DOPA/UWA-121 10 mg/kg, compared to 192 ± 14 min after L-DOPA/vehicle treatment (40% increase, $P < 0.01$, Tukey's *post hoc* test), 212 ± 17 min in the L-DOPA/UWA-121 1 mg/kg (26% increase, $P < 0.05$, Tukey's *post hoc* test), and 213 ± 13 min in the L-DOPA/UWA-121 3 mg/kg (26% increase, $P < 0.05$, Tukey's *post hoc* test).

In combination with L-DOPA, UWA-121 (10 mg/kg) significantly extended duration of ON-time without dyskinesia when compared to L-DOPA/vehicle and L-DOPA administered with lower doses of UWA-121 (1 and 3 mg/kg) treatments ($F(5, 15) = 5.094$, $P < 0.05$, one-way RM ANOVA, Fig. 4B). Thus, duration of ON-time without dyskinesia was 82 ± 19 min in the L-DOPA/UWA-121 10 mg/kg treatment, compared to 25 ± 11 min in the L-DOPA/vehicle treatment (215% increase, $P < 0.05$, Tukey's *post hoc* test), 30 ± 9 min in the L-DOPA/UWA-121 1 mg/kg treatment (173% increase, $P < 0.05$, Tukey's *post hoc* test), and 32 ± 12 min in the L-DOPA/UWA-121 3 mg/kg treatment (156% increase, $P < 0.05$, Tukey's *post hoc* test).

Table 4
UWA-121/122 monoamine re-uptake profiles.

	Noradrenaline	5-HT	Dopamine
UWA-121	>50,000	4640 ± 880	592 ± 130
UWA-122	>50,000	340 ± 67	>50,000

The affinity is provided as the mean Ki (nM) ± SEM of three independent experiments, each performed in triplicate.
5-HT: serotonin.

When administered with L-DOPA, UWA-121 10 mg/kg also extended (though non-significantly) duration of ON-time without disabling dyskinesia when compared to L-DOPA/vehicle treatment ($F(5, 15) = 1.237$, $P > 0.05$, one-way RM ANOVA, Fig. 4C). Thus, duration of ON-time without disabling dyskinesia was 138 ± 20 min in the L-DOPA/vehicle treatment, compared to 192 ± 31 min in the L-DOPA/UWA-121 10 mg/kg treatment (39% increase, $P > 0.05$, Tukey's *post hoc* test).

The combination of UWA-121 (1, 3 and 10 mg/kg) with L-DOPA had no effect on duration of ON-time with dyskinesia ($F(5, 15) = 0.8366$, $P > 0.05$, one-way RM ANOVA, Fig. 4D) or ON-time with disabling dyskinesia ($F(5, 15) = 0.3639$, $P > 0.05$, one-way RM ANOVA, Fig. 4E).

Unlike UWA-121, combining UWA-122 (1, 3 and 10 mg/kg) with L-DOPA did not affect duration of ON-time ($F(5, 15) = 1.318$, $P > 0.05$, one-way RM ANOVA, Fig. 4F), ON-time without dyskinesia ($F(5, 15) = 0.8049$, $P > 0.05$, one-way RM ANOVA, Fig. 4G), ON-time without disabling dyskinesia ($F(5, 15) = 0.1349$, $P > 0.05$, one-way RM ANOVA, Fig. 4H), ON-time with dyskinesia ($F(5, 15) = 1.680$, $P > 0.05$, one-way RM ANOVA, Fig. 4I), or ON-time with disabling dyskinesia ($F(5, 15) = 2.143$, $P > 0.05$, one-way RM ANOVA, Fig. 4J) when compared to L-DOPA/vehicle treatment.

3.4. UWA-121 does not exacerbate the severity of L-DOPA-induced dyskinesia

As illustrated in Fig. 5A, administering UWA-121 (1, 3 and 10 mg/kg) with L-DOPA had no effect on the severity of L-DOPA-induced dyskinesia when compared to L-DOPA/vehicle treatment ($F_{time}(5, 120) = 0.00$, $P = 1.00$, $F_{treatment}(3, 120) = 0.8997$, $P > 0.05$, and $F_{interaction}(15, 120) = 0.8480$, $P > 0.05$, two-way ANOVA). Accordingly, the severity of peak dose dyskinesia was not exacerbated in any of the L-DOPA/UWA-121 (1, 3 and 10 mg/kg) treatments when compared to L-DOPA/vehicle treatment (Friedman statistic (FS) = 1.707, $P > 0.05$, Friedman test, Fig. 5B).

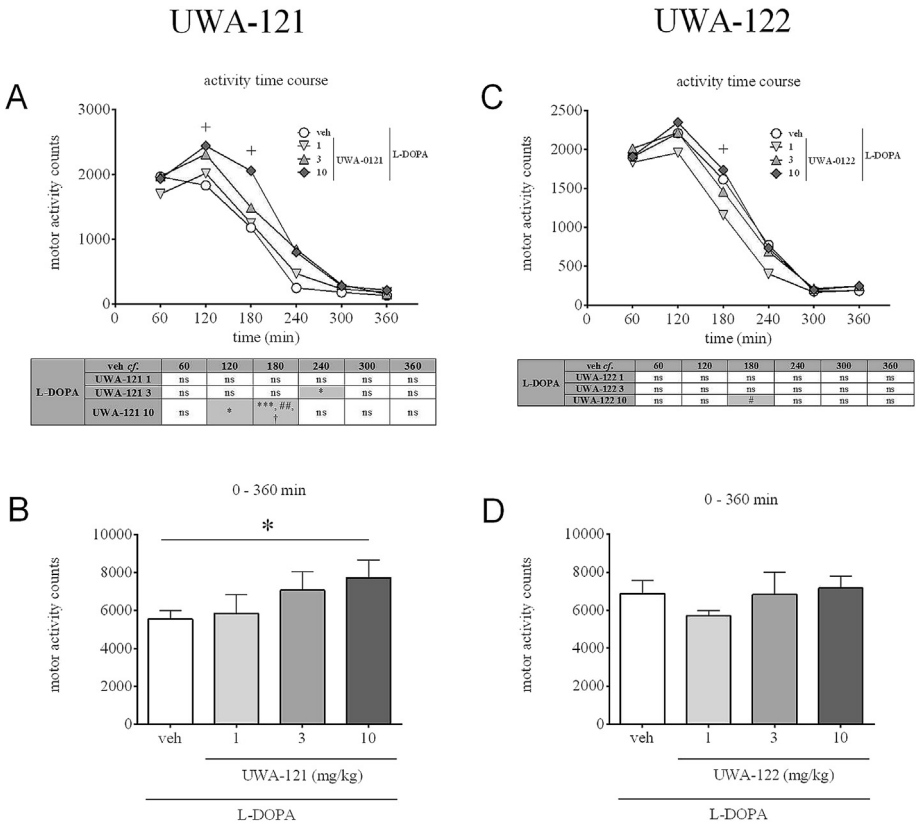


Fig. 3. Motor activity. A) Time course of motor activity in marmosets treated with L-DOPA and UWA-121 (1, 3 and 10 mg/kg) or vehicle. When added to L-DOPA, UWA-121 (10 mg/kg) significantly increased motor activity from 60 to 180 min when compared to L-DOPA/vehicle treatment ($P < 0.05$ from 60 to 120 min and $P < 0.001$ from 120 to 180 min). From 120 to 180 min, motor activity in the L-DOPA/UWA-121 10 mg/kg treatment was also significantly higher than in the L-DOPA/UWA-121 1 and 3 mg/kg treatments ($P < 0.01$ when compared to 1 mg/kg and $P < 0.05$ when compared to 3 mg/kg). From 180 to 240 min, the activity was significantly higher in the L-DOPA/UWA-121 10 mg/kg than in the L-DOPA/vehicle treatment ($P < 0.05$). B) Motor activity for the 0–360 min observation period (whole experiment) in marmosets treated with L-DOPA and UWA-121 (1, 3 and 10 mg/kg) or vehicle. In combination with L-DOPA, UWA-121 (10 mg/kg) significantly increased motor activity when compared to L-DOPA/vehicle treatment ($P < 0.05$). C) Time course of motor activity in marmosets treated with L-DOPA and UWA-122 (1, 3 and 10 mg/kg) or vehicle. From 120 to 180 min, motor activity was significantly lower in the L-DOPA/UWA-122 1 mg/kg treatment, when compared to L-DOPA/UWA-122 10 mg/kg treatment ($P < 0.05$ from 120 to 180 min). D) Motor activity for the 0–360 min observation period (whole experiment) in marmosets treated with L-DOPA and UWA-122 (1, 3 and 10 mg/kg) or vehicle. The addition of UWA-122 to L-DOPA did not modify motor activity when compared to L-DOPA/vehicle treatment ($P > 0.05$). *: $P < 0.05$ when compared to L-DOPA/vehicle; ***: $P < 0.001$ when compared to L-DOPA/vehicle; #: $P < 0.05$ when compared to L-DOPA/UWA-121-122 1 mg/kg; ##: $P < 0.01$ when compared to L-DOPA/UWA-121-122 1 mg/kg; †: $P < 0.05$ when compared to L-DOPA/UWA-121-122 3 mg/kg. Values are presented as the mean (A, C) or the mean ± SEM (B, D). For graphs A and C, the dots represent the mean motor activity counts for the preceding 60 min period. The crosses on the time course graphs (A, C) indicate time points for which there is significance. Data were analysed by two-way RM ANOVA followed by Bonferroni's *post hoc* tests (A, C) and by one-way RM ANOVA followed by Dunnett's *post hoc* tests (B, D).

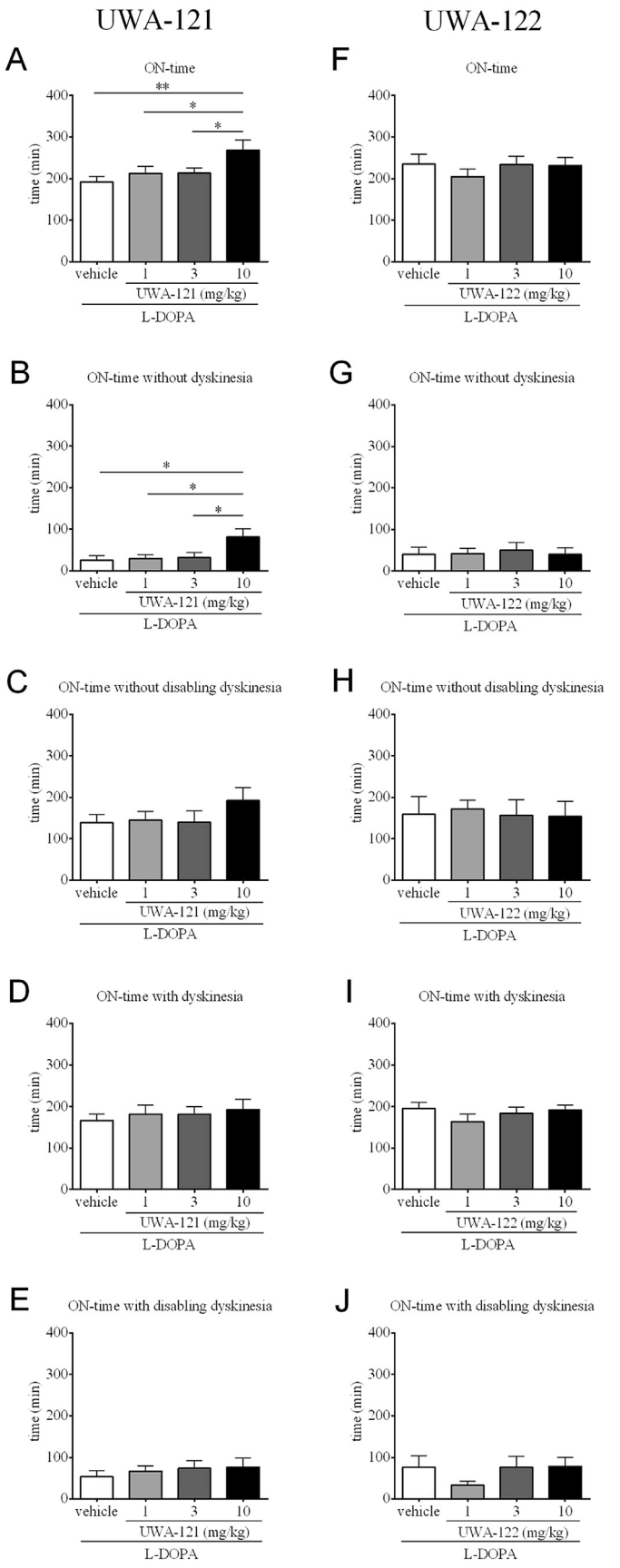
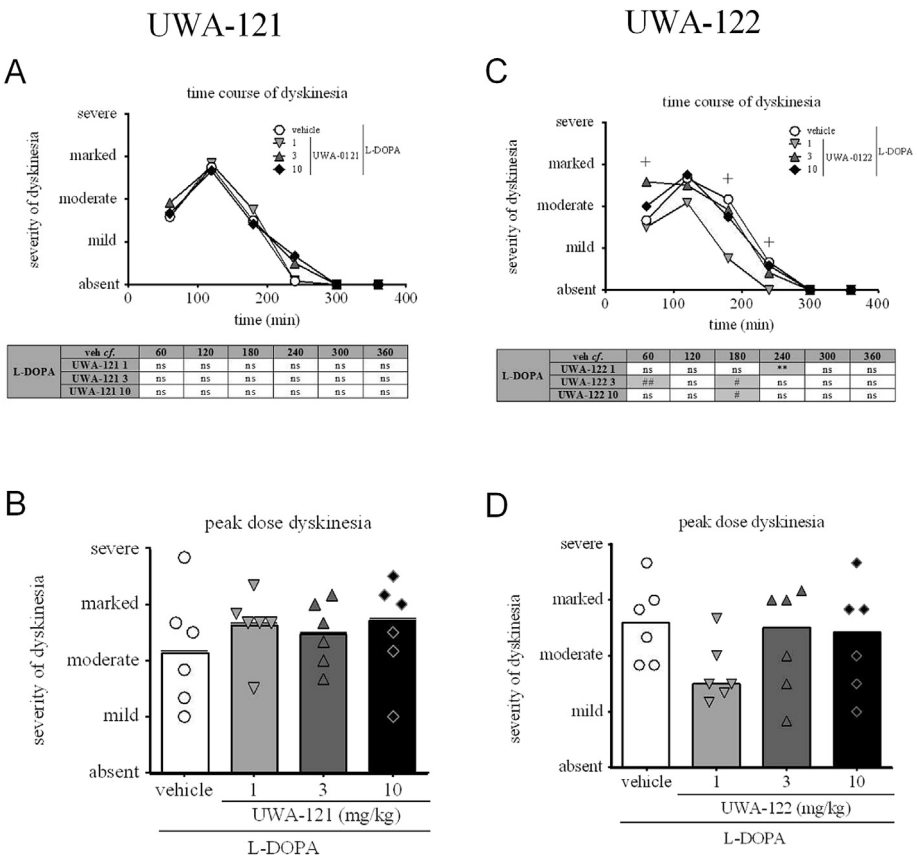


Fig. 4. ON-time and quality of ON-time. A) Duration of ON-time in marmosets treated with L-DOPA and UWA-121 (1, 3 and 10 mg/kg) or vehicle. Combining UWA-121 10 mg/kg with L-DOPA significantly extended duration of ON-time when compared to L-DOPA/vehicle ($P < 0.01$) and L-DOPA/UWA-121 1 and 3 mg/kg treatments ($P < 0.05$ for both). B) Duration of ON-time without dyskinesia in marmosets treated with L-DOPA and UWA-121 (1, 3 and 10 mg/kg) or vehicle. When added to L-DOPA, UWA-121 10 mg/kg significantly extended duration of ON-time without dyskinesia when compared to L-DOPA/vehicle and L-DOPA/UWA-121 1 and 3 mg/kg treatments ($P < 0.05$ for all). C) Duration of ON-time without disabling dyskinesia in marmosets treated with L-DOPA and UWA-121 (1, 3 and 10 mg/kg) or vehicle. The addition of UWA-121 to L-DOPA did not significantly increase duration of ON-time without disabling dyskinesia when compared to L-DOPA/vehicle treatment ($P > 0.05$). D) Duration of ON-time with dyskinesia in marmosets treated with L-DOPA and UWA-121 (1, 3 and 10 mg/kg) or vehicle. In combination with L-DOPA, UWA-121 did not modify duration of ON-time with dyskinesia when compared to L-DOPA/vehicle treatment ($P > 0.05$). E) Duration of ON-time with disabling dyskinesia in marmosets treated with L-DOPA and UWA-121 (1, 3 and 10 mg/kg) or vehicle. Adding UWA-121 to L-DOPA did not modify duration of ON-time with disabling dyskinesia when compared to L-DOPA/vehicle treatment ($P > 0.05$). F) Duration of ON-time in marmosets treated with L-DOPA and UWA-122 (1, 3 and 10 mg/kg) or vehicle. The addition of UWA-122 to L-DOPA did no modify duration of ON-time when compared to L-DOPA/vehicle treatment ($P > 0.05$). G) Duration of ON-time without dyskinesia in marmosets treated with L-DOPA and UWA-122 (1, 3 and 10 mg/kg) or vehicle. When added to L-DOPA, UWA-122 did no modify duration of ON-time without dyskinesia when compared to L-DOPA/vehicle treatment ($P > 0.05$). H) Duration of ON-time without disabling dyskinesia in marmosets treated with L-DOPA and UWA-122 (1, 3 and 10 mg/kg) or vehicle. Combining UWA-122 to L-DOPA did not modify duration of ON-time without disabling dyskinesia when compared to L-DOPA/vehicle treatment ($P > 0.05$). I) Duration of ON-time with dyskinesia in marmosets treated with L-DOPA and UWA-122 (1, 3 and 10 mg/kg) or vehicle. In combination with L-DOPA, UWA-122 did not modify duration of ON-time with dyskinesia when compared to L-DOPA/vehicle treatment ($P > 0.05$). J) Duration of ON-time with disabling dyskinesia in marmosets treated with L-DOPA and UWA-122 (1, 3 and 10 mg/kg) or vehicle. The addition of UWA-122 to L-DOPA did not modify duration of ON-time with disabling dyskinesia when compared to L-DOPA/vehicle treatment ($P > 0.05$). *: $P < 0.05$; **: $P < 0.01$. Values are presented as the mean ± SEM. Data were analysed by one-way ANOVA followed by Tukey's *post hoc* tests.

The combination of UWA-122 (1 mg/kg) and L-DOPA exerted a mild anti-dyskinetic effect when compared to L-DOPA/vehicle treatment or L-DOPA in combination with higher doses of UWA-122 (3, 10 mg/kg) treatment ($F_{time}(5, 120) = 0.00$, $P = 1.00$, $F_{treatment}(3, 120) = 8.875$, $P < 0.001$, and $F_{interaction}(15, 120) = 1.597$, $P > 0.05$, two-way ANOVA, Fig. 5C). Thus, from 180 to 240 min, severity of dyskinesia was reduced when L-DOPA/UWA-122 1 mg/kg was compared to L-DOPA/vehicle ($P < 0.01$, Bonferroni's *post hoc* test). From 120 to 180 min, severity of dyskinesia was reduced when L-DOPA/UWA-122 1 mg/kg was compared to L-DOPA/UWA-122, 3 and 10 mg/kg ($P < 0.05$ for both, Bonferroni's *post hoc* test). Severity of dyskinesia was also reduced from 0 to 60 min when L-DOPA/UWA-122 1 mg/kg was compared to L-DOPA/UWA-122 3 mg/kg ($P < 0.01$, Bonferroni's *post hoc* test). However, despite a trend, combining UWA-122 (1 mg/kg) and L-DOPA had no significant effect on the severity of peak dose dyskinesia when compared to L-DOPA/vehicle (FS = 6.056, $P = 0.1081$, Friedman test, Fig. 5D).

3.5. UWA-121 increases the duration of ON-time without psychosis-like behaviours

Neither UWA-121 nor UWA-122 exerted a deleterious effect on the severity of L-DOPA-induced psychosis-like behaviours. Thus, at no time during the 6 h observation period did the combination of UWA-121 (1, 3 and 10 mg/kg) and L-DOPA exacerbate the severity of psychosis-like behaviours when compared to L-DOPA/vehicle ($F_{time}(5, 120) = 0.00$, $P = 1.00$, $F_{treatment}(3, 120) = 0.7246$, $P > 0.05$, and $F_{interaction}(15, 120) = 0.9372$, $P > 0.05$, two-way ANOVA, Fig. 6A). Accordingly, in combination with L-DOPA, UWA-121 did not exacerbate the severity of peak dose psychosis-like behaviours when compared to L-DOPA/vehicle treatment (FS = 5.632, $P > 0.05$, Friedman test, Fig. 6B). Similarly, at no time did the addition of UWA-122 to L-DOPA exacerbate the severity of psychosis-like behaviours when compared to L-DOPA/vehicle ($F_{time}(5, 120) = 0.00$, $P = 1.00$, $F_{treatment}(3, 120) = 1.757$, $P > 0.05$, and $F_{interaction}(15,$



L-DOPA	veh cf.	60	120	180	240	300	360
	UWA-121 1	ns	ns	ns	ns	ns	ns
	UWA-121 3	ns	ns	ns	ns	ns	ns
	UWA-121 10	ns	ns	ns	ns	ns	ns

L-DOPA	veh cf.	60	120	180	240	300	360
	UWA-122 1	ns	ns	ns	**	ns	ns
	UWA-122 3	##	ns	#	ns	ns	ns
	UWA-122 10	ns	ns	#	ns	ns	ns

Fig. 5. Dyskinesia. A) Time course of L-DOPA-induced dyskinesia in marmosets treated with L-DOPA and UWA-121 (1, 3 and 10 mg/kg) or vehicle. At no time during the experiment did the addition of UWA-121 to L-DOPA alter the severity of dyskinesia when compared to L-DOPA/vehicle treatment ($P > 0.05$). B) Peak dose L-DOPA-induced dyskinesia in marmosets treated with L-DOPA and UWA-121 (1, 3 and 10 mg/kg) or vehicle. In combination with L-DOPA, UWA-121 did not modify the severity of peak dose dyskinesia when compared to L-DOPA/vehicle treatment ($P > 0.05$). C) Time course of L-DOPA-induced dyskinesia in marmosets treated with L-DOPA and UWA-122 (1, 3 and 10 mg/kg) or vehicle. From 0 to 60 min, dyskinesia were significantly less severe in the L-DOPA/UWA-122 1 mg/kg treatment when compared to the L-DOPA/UWA-122 3 mg/kg treatment ($P < 0.01$). Dyskinesia were also significantly less severe in the L-DOPA/UWA-122 1 mg/kg treatment when compared to the L-DOPA/UWA-122 3 mg/kg and 10 mg/kg treatments from 120 to 180 min ($P < 0.05$ for both), and when L-DOPA/UWA-122 1 mg/kg was compared to the L-DOPA/vehicle treatment from 180 to 240 min ($P < 0.01$). D) Peak dose L-DOPA-induced dyskinesia in marmosets treated with L-DOPA and UWA-122 (1, 3 and 10 mg/kg) or vehicle. Combining UWA-122 with L-DOPA did not modify the severity of peak dose dyskinesia when compared to L-DOPA/vehicle treatment ($P > 0.05$). **: $P < 0.01$ when compared to L-DOPA/vehicle; #: $P < 0.05$ when compared to L-DOPA/UWA-121-122 1 mg/kg; ##: $P < 0.01$ when compared to L-DOPA/UWA-121-122 1 mg/kg. For graphs A and C, the dots represent the median dyskinesia score for the preceding 60 min period. For graphs B and D, the bars represent the median score for dyskinesia, and the dots represent the individual score of each animal. For all graphs, the maximal possible score (most severe disability) was 24 and on the *y*-axis, mild = 6, moderate = 12, marked = 18, severe = 24. In A and C, following ranking, data were analysed by two-way ANOVA followed by Bonferroni's *post hoc* tests. In B and D, data were analysed by Friedman followed by Dunn's *post hoc* tests.

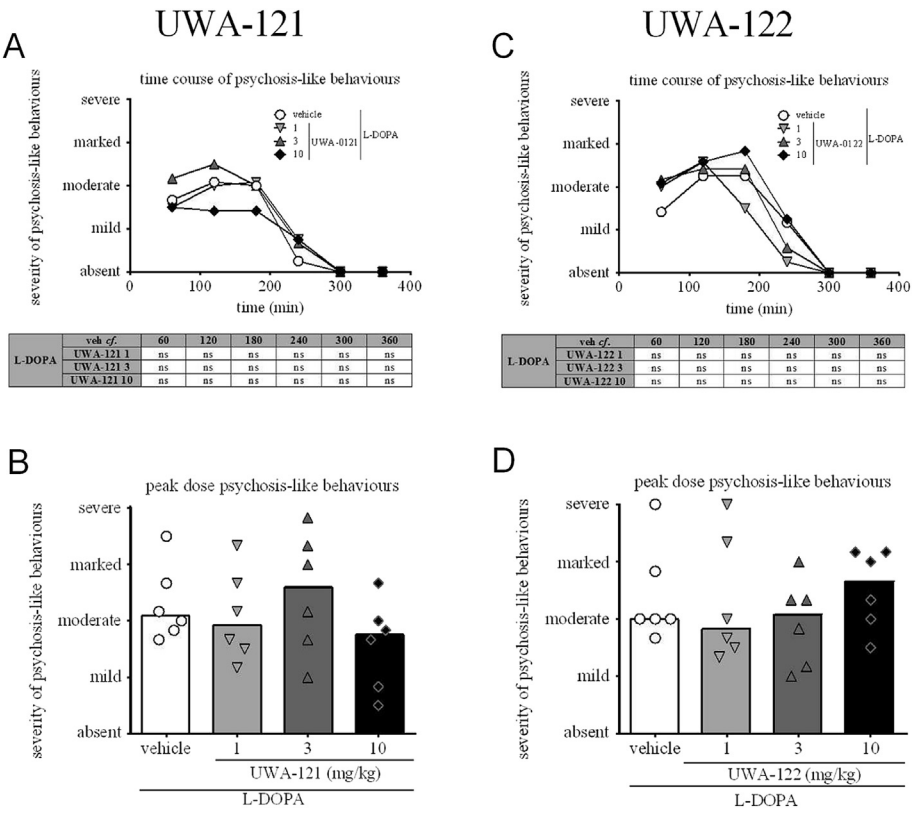
120) = 1.154, $P > 0.05$, two-way ANOVA, Fig. 6C). The severity of peak dose psychosis-like behaviours in the L-DOPA/UWA-122 treated animals (1, 3 and 10 mg/kg) was not different to the L-DOPA/vehicle treatment (FS = 1.821, $P > 0.05$, Friedman test, Fig. 6D).

Co-administration of UWA-121 (1, 3 and 10 mg/kg) with L-DOPA did not modify duration of ON-time with psychosis-like behaviours ($F(5, 15) = 0.4214$, $P > 0.05$, one-way RM ANOVA, Fig. 7A) or duration of ON-time with disabling psychosis-like behaviours ($F(5, 15) = 0.8453$, $P > 0.05$, one-way RM ANOVA, Fig. 7B) when compared to L-DOPA/vehicle treatment. However, combining UWA-121 (10 mg/kg) with L-DOPA significantly increased duration of ON-time without psychosis-like behaviours when compared to L-DOPA/vehicle treatment (22 ± 8 min *vs* 98 ± 20 min, a 345% increase, $F(5, 15) = 7.943$, $P < 0.01$, one-way RM ANOVA, $P < 0.01$, Tukey's *post hoc* test, Fig. 7C) and L-DOPA/UWA-121 1 and 3 mg/kg treatments, in which duration of ON-time without psychosis-like behaviours was 48 ± 16 min and 33 ± 6 min (increases of 104% and 196%, $P < 0.05$ and $P < 0.01$, respectively, Tukey's *post hoc* test).

The addition of UWA-122 (1, 3 and 10 mg/kg) to L-DOPA did not affect duration of ON-time with psychosis-like behaviours ($F(5, 15) = 1.342$, $P > 0.05$, one-way RM ANOVA, Fig. 7D), duration of ON-time with disabling psychosis-like behaviours ($F(5, 15) = 1.206$, $P > 0.05$, one-way RM ANOVA, Fig. 7E) or duration of ON-time without psychosis-like behaviours when compared to L-DOPA/vehicle treatment ($F(5, 15) = 0.2109$, $P > 0.05$, one-way RM ANOVA, Fig. 7F).

4. Discussion

In this study we have synthesised two novel monoamine re-uptake inhibitors, UWA-121 and UWA-122, characterised their pharmacological profiles, and assessed their behavioural effects in the MPTP-lesioned common marmoset. Specifically, duration of ON-time, severity of L-DOPA-induced dyskinesia and psychosis-like behaviours, have been investigated. UWA-121, was found to be a dual DAT > SERT inhibitor and significantly increased duration of ON-time, the majority of which is of "good quality", as duration of ON-time without dyskinesia and ON-time without psychosis-like behaviours were both extended. The extension in ON-time achieved with UWA-121 was not accompanied by any increase in dyskinesia severity. In contrast, UWA-122 was found to be a selective SERT inhibitor and had no effect on duration of ON-time,



L-DOPA	veh *cf.*	60	120	180	240	300	360
	UWA-121 1	ns	ns	ns	ns	ns	ns
	UWA-121 3	ns	ns	ns	ns	ns	ns
	UWA-121 10	ns	ns	ns	ns	ns	ns

L-DOPA	veh *cf.*	60	120	180	240	300	360
	UWA-122 1	ns	ns	ns	ns	ns	ns
	UWA-122 3	ns	ns	ns	ns	ns	ns
	UWA-122 10	ns	ns	ns	ns	ns	ns

Fig. 6. Psychosis-like behaviours. A) Time course of L-DOPA-induced psychosis-like behaviours in marmosets treated with L-DOPA and UWA-121 (1, 3 and 10 mg/kg) or vehicle. At no time during the experiment did the addition of UWA-121 to L-DOPA alter the severity of psychosis-like behaviours when compared to L-DOPA/vehicle treatment ($P > 0.05$). B) Peak dose L-DOPA-induced psychosis-like behaviours in marmosets treated with L-DOPA and UWA-121 (1, 3 and 10 mg/kg) or vehicle. Combining UWA-121 with L-DOPA did not modify the severity of peak dose psychosis-like behaviours when compared to L-DOPA/vehicle treatment ($P > 0.05$). C) Time course of L-DOPA-induced psychosis-like behaviours in marmosets treated with L-DOPA and UWA-122 (1, 3 and 10 mg/kg) or vehicle. At no time during the experiment did the addition of UWA-122 to L-DOPA alter the severity of psychosis-like behaviours when compared to L-DOPA/vehicle treatment ($P > 0.05$). D) Peak dose L-DOPA-induced psychosis-like behaviours in marmosets treated with L-DOPA and UWA-122 (1, 3 and 10 mg/kg) or vehicle. Simultaneous administration of UWA-122 and L-DOPA did not modify the severity of peak dose psychosis-like behaviours when compared to L-DOPA/vehicle treatment ($P > 0.05$). For graphs A and C, the dots represent the median psychosis-like behaviours score for the preceding 60 min period. For graphs B and D, the bars represent the median score for psychosis-like behaviours and the dots represent the individual score of each animal. For all graphs, the maximal possible score (most severe disability) was 24 and on the *y*-axis, mild = 6, moderate = 12, marked = 18, severe = 24. In A and C, following ranking, data were analysed by two-way ANOVA followed by Bonferroni's *post hoc* tests. In B and D, data were analysed by Friedman followed by Dunn's *post hoc* tests.

though it did significantly reduce severity of L-DOPA-induced dyskinesia at low dose. Why only the lowest dose of UWA-122 was effective at reducing dyskinesia is unclear at the moment.

Although the pharmacological characterisation of UWA-121/122 was performed in rat synaptosomal preparations, based on the literature, the rat DAT/SERT assay is likely to be predictive for the marmoset/human DAT/SERT potency. Thus, there seems to be a high correlation ($r = 0.998$) between the potency of several DAT inhibitors, such as mazindol, GBR-12,909 and amfonelic acid, in rat and in human synaptosomal membranes (Giros et al., 1992). Moreover, in rat and human synaptosomal preparations, citalopram, fluvoxamine, duloxetine, fluoxetine, etc. all exhibit similar pIC_{50} values at the SERT (Mantovani et al., 2009). In addition, duloxetine selectively inhibited the SERT at similar plasma concentration in rat and human (Bourdet et al., 2012). Based on these results from the literature, the rat DAT/SERT assay is likely to be predictive for the marmoset/human DAT/SERT potency.

Dopamine re-uptake inhibitors represent promising compounds in the treatment of PD and, as such, have been extensively studied both as monotherapy and, as here, as potential adjuncts to L-DOPA, to enhance its anti-parkinsonian benefits, and address the problem of wearing-off (Bara-Jimenez et al., 2004; Bedard et al., 1977; Hauser et al., 2007; Rascol et al., 2008; Teychenne et al., 1976). However, to date, no DAT inhibitor has shown robust efficacy as an adjunct to L-DOPA, without exacerbating L-DOPA-induced complications. We have proposed that this may in part result from an incomplete understanding of the pharmacology with respect to selectivity at DAT *versus* actions at other monoamine re-uptake sites. As mentioned in the introduction, for example, the triple monoamine re-uptake inhibitor, tesofensine, worsened dyskinesia in the context of a Phase II study (Rascol et al., 2008). The evaluation of clinically-relevant parameters at the pre-clinical level, to define not only efficacy but potential propensity to exacerbate L-DOPA-induced complications, may be of the utmost importance in drug candidate selection. Such measures would improve the chances of successful translation into the clinic, and also minimise the allocation of social resources and the exposure of patients to potentially ineffective or harmful compounds.

In a series of experiments, we have studied three dual DAT/SERT inhibitors, the S-enantiomer of MDMA (S-MDMA), UWA-101 and, as reported here, UWA-121; the relative affinities for DAT and SERT being SERT > DAT, DAT ≅ SERT and DAT > SERT, respectively. We have assessed not only the ability to extend ON-time but also propensity to exacerbate L-DOPA-induced dyskinesia and psychosis-like behaviours. The methodologies employed to assess these complications in non-human primates are the same across the three studies and have been validated as being predictive of clinical outcome, at least at Phase II (Fox et al., 2006a; Savola et al.,

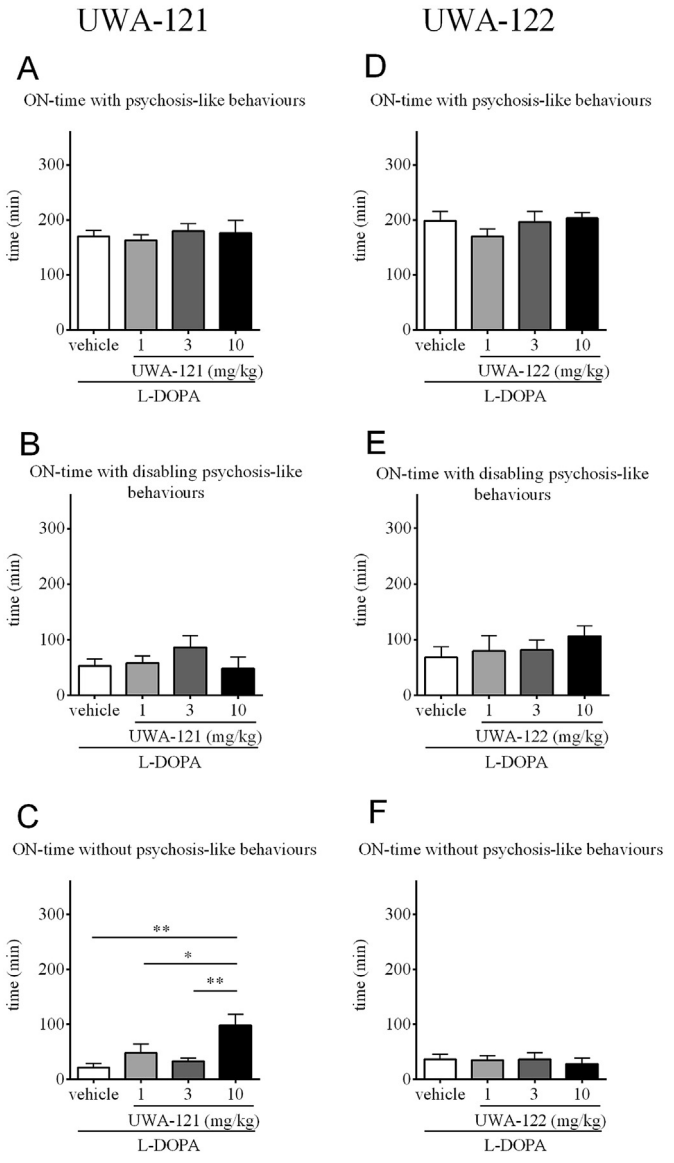


Fig. 7. ON-time with psychosis-like behaviours. A) Duration of ON-time with psychosis-like behaviours in marmosets treated with L-DOPA and UWA-121 (1, 3 and 10 mg/kg) or vehicle. Administration of UWA-121 with L-DOPA did not modify duration of ON-time with psychosis-like behaviours when compared to L-DOPA/vehicle treatment ($P > 0.05$). B) Duration of ON-time with disabling psychosis-like behaviours in marmosets treated with L-DOPA and UWA-121 (1, 3 and 10 mg/kg) or vehicle. Adding UWA-121 to L-DOPA did not modify duration of ON-time with disabling psychosis-like behaviours when compared to L-DOPA/vehicle treatment ($P > 0.05$). C) Duration of ON-time without psychosis-like behaviours in marmosets treated with L-DOPA and UWA-121 (1, 3 and 10 mg/kg) or vehicle. In combination with L-DOPA, UWA-121 (10 mg/kg) significantly extended duration of ON-time without psychosis-like behaviours when compared to L-DOPA/vehicle treatment ($P < 0.01$) and L-DOPA/UWA-121 1 and 3 mg/kg ($P < 0.05$ and $P < 0.01$, respectively). D) Duration of ON-time with psychosis-like behaviours in marmosets treated with L-DOPA and UWA-122 (1, 3 and 10 mg/kg) or vehicle. The addition of UWA-122 to L-DOPA did not modify duration of ON-time with psychosis-like behaviours when compared to L-DOPA/vehicle treatment ($P > 0.05$). E) Duration of ON-time with disabling psychosis-like behaviours in marmosets treated with L-DOPA and UWA-122 (1, 3 and 10 mg/kg) or vehicle. Combining UWA-122 with L-DOPA did not modify duration of ON-time with disabling psychosis-like behaviours when compared to L-DOPA/vehicle treatment ($P > 0.05$). F) Duration of ON-time without psychosis-like behaviours in marmosets treated with L-DOPA and UWA-122 (1, 3 and 10 mg/kg) or vehicle. When combined to L-DOPA, UWA-122 did not modify duration of ON-time without psychosis-like behaviours when compared to L-DOPA/vehicle treatment ($P > 0.05$). *: $P < 0.05$. Values are presented as the mean ± SEM. Data were analysed by one-way ANOVA followed by Tukey's *post hoc* tests.

2003). All three compounds extended duration of L-DOPA therapeutic action. However, S-MDMA (SERT > DAT) extended duration of L-DOPA anti-parkinsonian activity, but at the expense of exacerbating dyskinesia (Huot et al., 2011). UWA-101 (DAT ≅ SERT) extended duration of L-DOPA anti-parkinsonian action, without worsening dyskinesia severity, but exacerbated psychosis-like behaviours at high dose (Huot et al., 2012a; Johnston et al., 2012). Here we find that the DAT > SERT inhibitor UWA-121 extends duration of L-DOPA anti-parkinsonian action without exacerbating dyskinesia or psychosis-like behaviours.

UWA-101 is a racemic compound and we find that its activities do not reside solely in a single enantiomer, but are the composite of both (like MDMA) (Huot et al., 2011). Thus, UWA-121 provides both DAT and SERT inhibition, though with DAT > SERT, while UWA-122 provides potent and selective SERT inhibition. We propose that SERT inhibition is core to the ability of UWA-121, S-MDMA and UWA-101 to extend ON-time but that additional DAT actions are required; hence, UWA-122 does not extend ON-time. Serotonergic raphestriatal fibres contain the enzyme aromatic L-amino acid decarboxylase (Arai et al., 1996) and can thus convert L-DOPA into dopamine (Arai et al., 1995, 1994). Serotonergic fibres can also participate, via SERT, in the re-uptake of dopamine present in the synaptic cleft (Berger, 1978; Berger and Glowinski, 1978). Thus, SERT blockade could increase the concentration of synaptic dopamine generated from L-DOPA. However, such mechanisms are not sufficient to extend ON-time, as shown by UWA-122, and selective serotonin re-uptake inhibitors (SSRIs) in the clinical setting, and may even worsen parkinsonism (van de Vijver et al., 2002). We propose that the ability of UWA-121, as well as S-MDMA and UWA-101, to extend ON-time arises because of an additional inhibition of DAT. In the absence of DAT inhibition, the transporter on remaining dopamine terminals may be able to buffer rises in synaptic dopamine caused by SERT inhibition, explaining the requirement for concomitant DAT and SERT activity to unmask effects on SERT to enhance L-DOPA anti-parkinsonian action. It is, perhaps, surprising that DAT activity plays such a key role in a denervated state, as the MPTP regimens employed to elicit parkinsonism in the marmoset lead to greater than 80% reduction in striatal dopamine re-uptake and DAT binding (Ando et al., 2012; Jenner et al., 1984). These agents therefore have much reduced target, in comparison to the normal situation, upon which to act. However, our results suggest that there is sufficient DAT remaining in the striatum of the MPTP-lesioned primate such that its inhibition can elevate synaptic dopamine levels. This is also illustrated by a reversal of parkinsonism, comparable to the one obtained with L-DOPA, when the selective DAT inhibitor GBR-12,909 is administered as monotherapy to the parkinsonian non-human primate (Hansard et al., 2002). Whether UWA-121 would exert a similar action in the L-DOPA-treated MPTP-lesioned macaque, in which striatal dopamine denervation is greater than the marmoset (Fernagut et al., 2010; Huot et al., 2012b), remains to be determined. Clinically, studies where the dual DAT/NET inhibitor methylphenidate was administered to PD patients suggested that DAT/NET inhibition enhances L-DOPA anti-parkinsonian action, but may increase the number of patients exhibiting dyskinesia (Nutt et al., 2004). Moreover, the improvement in parkinsonian features upon methylphenidate administration was limited to specific parameters such as tapping speed and did not encompass extension of ON-time (Nutt et al., 2007, 2004). Similarly, the affinity of nomifensine and tesofensine at the NET might be why the two molecules exacerbated dyskinesia.

While both DAT and SERT inhibition appear to be necessary to extend duration of L-DOPA benefit, the ratio of DAT to SERT affinity seems to define the propensity to exacerbate L-DOPA-induced complications. When SERT affinity is relatively high, both

dyskinesia and psychosis are exacerbated. Given the discussion above, this might represent a situation where the highest levels, or widest fluctuations, of L-DOPA-derived dopamine are achieved in the synapse. In the presence of a relatively lower SERT to DAT inhibition ratio, as with UWA-121, more balanced and physiological synaptic levels of dopamine might be achieved, alleviating parkinsonism with less complications. Studies such as *in vivo* microdialysis to determine striatal dopamine levels, in dopamine-depleted animals, after administration of L-DOPA, in combination with compounds exhibiting different SERT to DAT affinity ratios, are required to test this hypothesis. Studies assessing the pharmacokinetic (PK) profile of each of UWA-101/121/122 are also required, to further the understanding of the mechanisms whereby these drugs exert their therapeutic effects and to determine, for instance, plasma levels that will facilitate translation to clinical trials. In addition, studies where higher doses of UWA-121 would be administered are necessary to further characterise the therapeutic potential of this drug, as the highest dose (10 mg/kg) was the only dose providing an anti-parkinsonian benefit, thereby precluding the determination of the full dose–response profile of the molecule.

In conclusion, we propose that dual DAT/SERT inhibitors have potential, as adjunct therapy, to enhance the anti-parkinsonian benefit of L-DOPA. In the MPTP-lesioned marmoset, where there is clearly sufficient remaining DAT for its inhibition to have functional relevance, UWA-121 strikes a balance of inhibition of DAT > SERT that provides an enhancement of anti-parkinsonian benefit that is not compromised by an exacerbation of dyskinesia or psychosis. However, it remains to be seen how the anti-parkinsonian efficacy of such drugs may change with evolution of disease, and loss of remaining DAT, in advanced PD. Whether the efficacy of this class of agents is maintained upon chronic administration will also have to be addressed in future studies.

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