

Title: L-745,870 reduces L-3,4-dihydroxyphenylalanine-induced dyskinesia in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned macaque model of Parkinson's disease

Authors: Philippe Huot, Tom H Johnston, James B Koprach, Ahmed Aman, Susan H Fox, Jonathan M Brotchie

Affiliations:

PH, THJ, JBK, SHF, JMB: Toronto Western Research Institute, University Health Network, 399 Bathurst Street, Toronto, Ontario, Canada, M5T 2S8

PH, SHF: Division of Neurology, Movement Disorder Clinic, Toronto Western Hospital, University Health Network, 399 Bathurst Street, Toronto, Ontario, Canada, M5T 2S8

AA: Ontario Institute for Cancer Research, MaRS Centre, 101 College Street, Toronto, Ontario, Canada, M5G 0A3

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Corresponding author: Jonathan M Brotchie: Toronto Western Research Institute, MCL 11-419, Toronto Western Hospital, 399 Bathurst Street, Toronto, Ontario, Canada, M5T 2S8, Fax: +1-416-603-5447, Phone: +1-416-603-5369, Email: brotchie@uhnres.utoronto.ca

or

Philippe Huot: Toronto Western Research Institute MP 12-303, Toronto Western Hospital, 399 Bathurst Street, Toronto, Ontario, Canada, M5T 2S8, Fax: +1-416-603-5447, Phone: +1-416-603-5800 ext 3472, Email: phuot@uhnres.utoronto.ca

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Non-standard abbreviations: 6-OHDA: 6-hydroxydopamine; AMPA: α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AUC: area under the curve; CL: clearance; GABA: gamma-aminobutyric acid; GP: globus pallidus; GPe: globus pallidus pars externa; GPi: globus pallidus pars interna; k_{el} : elimination rate constant; K_i : inhibition constant; L-DOPA: L-3,4-dihydroxyphenylalanine; MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine); NIH: National Institute of Health; NMDA: *N*-methyl-D-aspartate; PK: pharmacokinetics; SNc: substantia nigra pars compacta; SNr: substantia nigra pars reticulata; STN: subthalamic nucleus; $t_{1/2}$: half-life; t_{max} : time to maximal concentration

Abstract

L-3,4-dihydroxyphenylalanine (L-DOPA)-induced dyskinesia remain an unmet challenge in the treatment of Parkinson's disease (PD). Here, we investigate the potential anti-dyskinetic efficacy of L-745,870, a potent and selective dopamine D₄ receptor antagonist with a good toxicology profile and an excellent safety and tolerability record in Phase I/II clinical studies, for non-PD indications. Six macaques were rendered parkinsonian by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration. After induction of stable and marked dyskinesia, animals were administered acute challenges of L-745,870 in combination with L-DOPA. To guarantee D₄ selectivity at the doses employed in the study, we determined plasma, cerebrospinal fluid (CSF) and brain levels of L-745,870. Co-administration of L-745,870 (1 mg/kg) and L-DOPA significantly reduced the severity of dyskinesia, by up to 59%, in comparison to L-DOPA alone ($P < 0.01$). L-745,870 had no effect on duration of anti-parkinsonian benefit, ON-time ($P > 0.05$). However, L-745,870 (1 mg/kg) significantly increased duration of ON-time without disabling dyskinesia (+204%, $P < 0.001$) and decreased duration of ON-time with disabling dyskinesia when compared to L-DOPA alone (-56%, $P < 0.01$). Brain levels of L-745,870 (~600 ng/g) were within the range at which L-745,870 provides selective D₄ receptor antagonism. Plasma levels were comparable to those demonstrated to be well-tolerated in human studies. These data suggest that selective D₄ receptor antagonists represent a potential therapeutic approach for L-DOPA-induced dyskinesia. Importantly, L-745,870 has already undergone significant clinical development, has an excellent profile for a therapeutic candidate, and could be advanced rapidly to Phase IIa clinical studies for dyskinesia in PD.

Introduction

The classic manifestations of Parkinson's disease (PD) are caused by the degeneration of dopaminergic neurons of the substantia nigra, which leads to a deficit in dopamine within the striatum (Hassler, 1938; Ehringer and Hornykiewicz, 1960). As such, dopamine replacement therapy with the dopamine precursor L-3,4-dihydroxyphenylalanine (L-DOPA), is the most effective treatment for PD (Fahn, 2008). However, chronic treatment with L-DOPA is marred by the development of abnormal involuntary movements, dyskinesia (Hely et al., 2005; Fabbrini et al., 2007), which negatively impact on quality of life (Pechevis et al., 2005).

While the involvement of D₁, D₂ and D₃ receptors in dyskinesia has been extensively studied (Gold et al., 2007; Guigoni et al., 2007; Dupre et al., 2008; Jenner, 2008; Visanji et al., 2009a), D₄ receptors have been relatively neglected, even though they are present within the basal ganglia, key structures involved in both parkinsonism and dyskinesia (DeLong, 1990; DeLong and Wichmann, 2007). Thus D₄ receptors are encountered within the striatum (Rivera et al., 2002), pallidum (Mauger et al., 1998) and subthalamic nucleus (Flores et al., 1999), and could therefore play an important role in basal ganglia signalling.

In addition to their localisation within the basal ganglia, pharmacological studies hint that D₄ receptors might represent a promising therapeutic target for L-DOPA-induced dyskinesia. Thus, sarizotan is a molecule that effectively alleviated L-DOPA-induced dyskinesia in two experimental models of parkinsonism, the 6-hydroxydopamine (6-OHDA)-lesioned rat (Marin et al., 2009) and the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned non-human primate (NHP) (Gregoire et al., 2009) as well as in Phase II studies (Olanow et al., 2004; Bara-Jimenez et al., 2005). Unfortunately, sarizotan was not superior to placebo in Phase III studies (Goetz et al., 2008). However, while the therapeutic effects of sarizotan are generally attributed to an agonist action at serotonergic type 1A (5-

HT_{1A}) receptors, sarizotan is essentially equipotent as an antagonist at dopamine D₄ receptors (Bartoszyk et al., 2004), and a positron emission tomography study has demonstrated that therapeutically-relevant doses of sarizotan lead to equivalent occupancy of 5-HT_{1A} and D₂-like receptors (Rabiner et al., 2002), thereby highlighting the potentially important contribution of D₂-like receptors in the mechanism of action of sarizotan. In an early attempt to address the issue of D₄ involvement in L-DOPA-induced dyskinesia, a study performed in the MPTP-lesioned NHP demonstrated a reduction in dyskinesia following treatment with the clozapine analogue JL-18 (Hadj Tahar et al., 2000). While JL-18 is a potent D₄ antagonist, it also exhibits high affinity for serotonergic type 2A (5-HT_{2A}), D₂ and muscarinic receptors, the D₄ selectivity *cf.* the next most potent target is at best 2-fold (Liegeois et al., 1995), and since no PK or receptor occupancy studies were performed, the role played by these non-D₄ receptors in the anti-dyskinetic action of JL-18 remains unclear.

L-745,870 (3-([4-(4-chlorophenyl)piperazin-1-yl]methyl)-1H-pyrrolo[2,3-b]pyridine) is a molecule that was developed to treat schizophrenia and its anti-psychotic efficacy was tested in a Phase IIa clinical trial (Bristow et al., 1997b; Kramer et al., 1997). L-745,870 has also been tested in the mouse, rat and NHP (Bristow et al., 1997a; Patel et al., 1997). L-745,870 is a potent and selective D₄ antagonist, exhibiting nearly 100-fold selectivity for D₄ receptors over its next target (Patel et al., 1997; Stewart et al., 2004; Nakane et al., 2005). Although the drug did not prove to be effective for the treatment of schizophrenia, invaluable data regarding its safety, tolerability and pharmacokinetic (PK) profile in human were gathered during the development process (Bristow et al., 1997b; Kramer et al., 1997). For instance, L-745,870 was well-tolerated by 26 schizophrenic patients receiving doses of 15 mg once daily for 4 weeks (Kramer et al., 1997). In that study, no extrapyramidal reaction or other adverse effects such as sedation were noted, plasma levels at which 90% of D₄ receptors are occupied and plasma half-life ($t_{1/2}$) were established (Kramer et al., 1997).

Antagonising D₄ receptors might thus represent a way to alleviate L-DOPA-induced dyskinesia, but the anti-dyskinetic efficacy of D₄ antagonists has yet to be demonstrated. In the present study, we have assessed the anti-dyskinetic efficacy of L-745,870 in the MPTP-lesioned macaque.

Materials and methods

Animals

Six cynomolgus macaques (*Macaca fascicularis*; three females and three males, 5.88 ± 1.02 kg, 8.5 ± 0.3 years) were obtained from Shared Animal Health (Beijing, PRC). Animals were housed individually in conditions of controlled temperature (23 ± 2°C), humidity (50 ± 2%) and light (12 h light/dark cycle, 07:00 lights on). Animals were group housed, housing conditions exceeded USA National Institutes of Health (NIH), Canadian Council on Animal Care and United Kingdom Home Office guidelines (2.4 m x 3.0 m x 1.0 m). Animals had unrestricted access to primate diet and water and fresh fruits were supplemented daily. Housing cages were enriched with auditory and tactile stimuli. Efforts were made to reduce to a minimum the number of animals required for statistically valid analyses and to minimise their suffering. Experiments were carried out in China with local institutional animal care and use committee approval and in accordance with the Guide for the Care and Use of Laboratory animals as adopted and promulgated by the USA NIH (NIH, 1996).

Induction of parkinsonism and dyskinesia in the cynomolgus macaque

Animals were rendered parkinsonian by once daily injection of MPTP hydrochloride (Sigma-Aldrich, Oakville, Canada) dissolved into 0.9% sterile NaCl (0.2 mg/kg), administered intravenously as previously described (Johnston et al., 2010a; Johnston et al., 2010b), until the first appearance of parkinsonism (mean cumulative dose 14.4 ± 5.4 mg). After stabilisation of the parkinsonian phenotype, dyskinesia, both choreiform and dystonic, were induced by chronic L-DOPA/ benserazide treatment (25/6.25 mg/kg p.o. twice daily, 4:1 ratio, administered as Madopar®, Hoffmann-La Roche Limited, Shanghai, PRC).

Administration of L-745,870 in combination with L-DOPA to MPTP-lesioned macaques

On days of behavioural assessment, at 09:00, macaques were administered L-DOPA/ benserazide orally (dose individually tailored for each animal, 34.2 ± 1.5 mg/kg, 4:1 L-DOPA/ benserazide ratio as Madopar®) in combination with either vehicle (0.00037% HCl in water) or L-745,870 trihydrochloride, (equivalent to 0.01, 0.1, 0.3 and 1.0 mg/kg of drug free base orally; Tocris Bioscience, Ellisville, USA). Drug administration schedule was randomised according to a Latin square design. After administration of treatment, each macaque was placed individually into an observation cage (1.5 × 1.0 × 1.1 m) containing food, water and primate toys, and left undisturbed for the 6 h duration of the experiment.

Motor activity was detected by an automated computer-based passive infrared activity monitoring and behaviour was recorded on DVD for *post hoc* analysis by a neurologist specialised in movement disorders blinded to the treatment given. At least 72 h were left between each treatment in any animal.

Behavioural assessment of L-745,870 in the MPTP-lesioned macaque

The scales used for assessment of behaviour were described in detail previously (Visanji et al., 2009b; Johnston et al., 2010a). 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 to 4 scale: 0 = walking on the floor and/ or climbing on the walls or roof of the cage; 4 = no movement. Bradykinesia was rated on a 0 to 3 scale: 0 = normal speed and initiation of movement; 3 = marked slowing, or unable to move, with prolonged freezing episodes. Posture was rated on a 0 to 2 scale: 0 = normal, upright; 2 = hunched body and neck, face down, may lose balance. Attention/ alertness was rated on a 0 to 1 scale: 0 = present, looking around, observing; 1 = absent. 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 derived, summing the scores of the aforementioned behaviours. The maximal parkinsonian disability score per 5 min observation period was 10.

Dyskinesia were scored, for 5 min every 10 min, on a 0 to 4 scale: 0 = absent; 1 = mild, fleeting, not interfering with normal activity, 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 disabling, *i.e.*, interfering with normal activity, present less than 70% of the observation period; and 4 = severe, continuous, disabling, replacing normal activity, present more than 70% of the observation period. Choreiform and dystonic dyskinesia were both assessed separately and the dyskinesia score attributed reflected the most disabling dyskinesia observed, whether chorea or dystonia.

Parkinsonian disability and dyskinesia scores were cumulated for each hour across the entire 6 h of observation. The duration of anti-parkinsonian benefit, *i.e.* ON-time, was defined as the number of minutes for which bradykinesia score was 0, based on widely used criteria

for clinical diagnosis of idiopathic PD (Hughes et al., 1992). ON-time was further divided as “good” or “bad” quality, depending on the severity of dyskinesia present. Thus, “good quality” ON-time was defined as the number of minutes when bradykinesia score was 0 and dyskinesia were either absent or non-disabling, *i.e.*, mild, or moderate in intensity (scores of 0, 1, 2), while “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, 4). ON-time without disabling dyskinesia was defined as the sum of ON-time without dyskinesia (score of 0) and ON-time with non-disabling dyskinesia (scores of 1, 2). These scales were developed to provide a NHP analogue of the measures such as ON-time and ON-time with troublesome dyskinesia that are widely used in clinical studies (Rascol et al., 2005).

Determination of L-745,870 levels in the plasma, cerebrospinal fluid and brain of the MPTP-lesioned macaque

Plasma pharmacokinetic study: administration of L-745,870 and blood sampling

In a series of studies, conducted independently from behavioural observations, in three MPTP-lesioned cynomolgus macaques, plasma levels of drug were assessed following a single administration of L-745,870 (0.1, 0.3 and 1 mg/kg p.o., as well as 0.3 mg/kg i.v.) given in combination with L-DOPA/ benserazide (30 mg/kg p.o.). Plasma levels of L-745,870 (0.3 mg/kg p.o.) were also assessed in the absence of L-DOPA, to determine if L-DOPA alters L-745,870 PK profile. All animals received all treatments once, in a non-randomised, ascending dose fashion. A minimum of 7 days were left between treatments in the same animal. 2.0 ml of venous blood were removed at each of the following timepoints: predose, 15 and 30 min and 1, 2, 3, 6, 8 and 24 h postdrug. Each blood sample was transferred into K⁺-EDTA-coated

tubes (Becton Dickinson, Mississauga, Canada), gently inverted, and centrifuged at $1500 \times g_{av}$ for 10 min at 4°C. Plasma layers were frozen and stored at -80°C until processing.

Cerebrospinal fluid and brain tissue collection

Three MPTP-lesioned cynomolgus macaques were administered L-745,870 (1 mg/kg p.o.) given in combination with L-DOPA/ benserazide (30 mg/kg p.o.). 75 min later, animals were administered an overdose of sodium pentobarbital (Euthanyl®; Bimeda – MTC Animal Health Inc, Cambridge, Canada). 15 min later (90 min after drug administration, corresponding to peak behavioural effect according to motor activity counts, see Figure 2A), animals were perfused transcardially with 0.9% NaCl. 2.0 ml of blood were collected for determination of plasma L-745,870 levels in order to correlate plasma, CSF and brain levels. 2.0 ml of CSF were collected from the cisterna magna and immediately stored at -80°C. Brain was rapidly removed and the primary motor cortex and putamen were dissected out, flash-frozen in isopentane and stored at -80°C until processing.

Assessment of L-745,870 levels via liquid chromatography – mass spectrometry/ mass spectrometry

Brain tissue was homogenised into a ddH₂O solution on ice by using an ultrasonic tissue homogeniser (Mandel Scientific Company Inc, Guelph, Canada). Aliquots of 45 µl of macaque biological samples (plasma, CSF or brain homogenate) were supplemented with 5 µl of methanol and 5 µl of mirtazapine solution (the internal standard, 100 ng/ml), were transferred into a 1.5 ml microcentrifuge Eppendorf tube and vortexed. After alkalisation with an additional 10 µl of alkaliser (ammonium chloride dissolved into ammonia water, pH 10), 1 ml of methyl *tert*-butyl ether (MTBE) was added into the microcentrifuge tube. Samples were then vortexed for 3 min and centrifuged at 14,000 revolutions per min (rpm)

for 3 min. Supernatant was then transferred to a 96-well plate. Extraction solutions were evaporated to dryness under a stream of nitrogen at room temperature. Residues were reconstituted with 100 μ l of methanol in ddH₂O (1:1 v/v) and transferred into injection vials. 10 μ l of each solution was injected for liquid chromatography (LC) – mass spectrometry (MS)/MS analysis.

Chromatographic separation was performed on a Shimadzu LC-10A VP system equipped with two binary pumps (Kyoto, Japan), a CTC-HTS autosampler (Zwingen, Switzerland) and a Kinetex PFP (2.1 \times 50 mm, 2.6 μ m, 100 Å) column (Rockford, USA). Chromatography was performed at 40°C with an isocratic gradient at 0.1% formic acid and 5 mM ammonium acetate in water and 0.1% formic acid in acetonitrile (10:90, v/v). Flow rate was set at 0.6 ml/min. The MS/MS system was an MDS Sciex API-4000 mass spectrometer with an electrospray ionisation probe (Toronto, Canada). Analytes were detected by multiple reaction monitoring in positive mode with a dwell time of 150 ms. MS/MS conditions were optimised by T-tube infusion of 100 ng/ml for the analyte in methanol:ddH₂O (1:1 v/v) at a flow rate of 20 μ l/min. The optimal transitions from the protonated molecular ion to a diagnostic fragment ion were mass to charge ratio (m/z) 327.2 \rightarrow 131.1 for L-745,870 and m/z 266.1 \rightarrow 195.1 for mirtazapine. Collision gas was nitrogen, collision energy was set at 21 V, the source temperature was 450°C and the ion spray voltage was set at 5,000 V.

Calibration curves (0.1 – 100 ng/ml) were generated and their linearity was fitted with the LC-MS/MS software Analyst version 1.4.1 (Applied Biosystems Inc, Foster City, USA). Concentrations of biological samples were calculated with the calibration curves and expressed as nmol/ml.

Statistical analysis

Continuous motor activity scores were analysed by one-way repeated measures analysis of variance (RM ANOVA) followed by Tukey's multiple comparison *post hoc* tests. Categorical, discontinuous scores for parkinsonian disability and dyskinesia severity were analysed using non-parametric Friedman's followed by Dunn's multiple comparison *post hoc* tests. Continuous ON-time data were analysed by one-way RM ANOVA followed by Tukey's multiple comparison *post hoc* tests. Time course data for motor activity counts were analysed by a two-way RM ANOVA followed by Bonferroni's multiple comparison *post hoc* tests. Time course data for parkinsonian disability and dyskinesia scores were ranked by macaque across each of the seven treatments and analysed by a two-way ANOVA followed by Bonferroni's multiple comparison *post hoc* tests. Levels of L-745,870 in the primary motor cortex and putamen were compared using parametric one-tailed Student's *t* test. PK parameters of L-745,870 administered with and without L-DOPA were compared using parametric one-tailed Student's *t* test. Statistical significance was assigned when $P < 0.05$. Statistical analyses were computed using GraphPad Prism 5.03 (GraphPad Software Inc, La Jolla, USA).

Determination of L-745,870 PK parameters, area under the curve (AUC), maximal plasma concentration (C_{\max}), time of maximal plasma concentration (t_{\max}), elimination rate constant (K_{el}), $t_{1/2}$, clearance (CL) and bioavailability was done using Microsoft Office Excel (Microsoft Corporation, Redmond, USA) and GraphPad Prism 5.03.

Results

L-745,870 reduces the severity of L-DOPA-induced dyskinesia and extends duration of “good quality” ON-time

Administration of L-745,870 1 mg/kg in combination with L-DOPA provided a significant reduction of L-DOPA-induced dyskinesia severity when compared to L-DOPA/vehicle treatment. Administration of L-745,870 also significantly extended duration of good-quality ON-time, while reducing duration of bad-quality ON-time.

Over the 360 min observation period, there was a significant effect of treatment and an interaction between treatment and time, but no effect of time alone on the severity of L-DOPA-induced dyskinesia ($F_{\text{time}}(5,210) = 0.0$, $P > 0.05$, $F_{\text{treatment}}(6,210) = 35.63$, $P < 0.001$, and $F_{\text{interaction}}(30,210) = 5.539$, $P < 0.001$, two-way ANOVA following ranking of data, Figure 1A). Thus, L-DOPA, in combination with vehicle or L-745,870, elicited significantly more severe dyskinesia than vehicle/ vehicle or vehicle/ L-745,870 treatments from 0-180 min following drug administration (all $P < 0.01$, Bonferroni's *post hoc* test). However, L-745,870 1 mg/kg significantly alleviated the severity of dyskinesia when compared to L-DOPA alone (by 47% from 0-60 min, $P < 0.01$, 30% from 60-120 min, $P < 0.001$ and by 59% from 120-180 min, $P < 0.01$, Bonferroni's *post hoc* test). L-745,870 1 mg/kg also reduced severity of dyskinesia when compared to lower doses of L-745,870 ($P < 0.01$ from 60-180 min when compared to L-DOPA/ L-745,870 0.01 mg/kg, and $P < 0.05$ from 120-180 min when compared to L-DOPA/ L-745,870 0.1 mg/kg, Bonferroni's *post hoc* test). Lower doses of L-745,870 did not alleviate L-DOPA-induced dyskinesia ($P > 0.05$, Bonferroni's *post hoc* test).

Duration of ON-time with dyskinesia was unchanged by the addition of L-745,870 ($F(5,30) = 48.99$, $P < 0.001$, one-way RM ANOVA; $P > 0.05$ when L-DOPA/ L-745,870, regardless of the dose of L-745,870, was compared to L-DOPA alone, Tukey's *post hoc* test, Figure 1B).

However, duration of ON-time with disabling dyskinesia was significantly reduced by combining L-745,870 1 mg/kg to L-DOPA ($F(5,30) = 33.08$, $P < 0.001$, one-way RM ANOVA, Figure 1C). Thus, duration of ON-time with disabling dyskinesia was 55 ± 15 min in the L-DOPA/ L-745,870 1 mg/kg treatment, compared to 127 ± 18 min in the L-DOPA/ vehicle treatment (56% decrease, $P < 0.001$, Tukey's *post hoc* test), 135 ± 13 min in the L-DOPA/ L-745,870 0.01 mg/kg treatment (59% decrease, $P < 0.001$, Tukey's *post hoc* test), 110 ± 10 min in the L-DOPA/ L-745,870 0.1 mg/kg treatment (50% decrease, $P < 0.05$, Tukey's *post hoc* test) and 100 ± 8 min in the L-DOPA/ L-745,870 0.3 mg/kg treatment (45% decrease, $P < 0.05$, Tukey's *post hoc* test).

Accordingly, duration of ON-time without disabling dyskinesia was significantly increased when L-745,870 1 mg/kg was added to L-DOPA ($F(5,30) = 12.43$, $P < 0.001$, one-way RM ANOVA, Figure 1D). Thus, duration of ON-time without disabling dyskinesia was 132 ± 30 min in the L-DOPA/ L-745,870 1 mg/kg treatment, compared to 43 ± 10 min in the L-DOPA/ vehicle treatment (204% increase, $P < 0.001$, Tukey's *post hoc* test), 33 ± 13 min in the L-DOPA/ L-745,870 0.01 mg/kg treatment (295% increase, $P < 0.001$, Tukey's *post hoc* test), 52 ± 13 min in the L-DOPA/ L-745,870 0.1 mg/kg treatment (155% increase, $P < 0.01$, Tukey's *post hoc* test), and 53 ± 13 min in the L-DOPA/ L-745,870 0.3 mg/kg treatment (147% increase, $P < 0.01$, Tukey's *post hoc* test).

Duration of ON-time without dyskinesia was also significantly extended by adding L-745,870 1 mg/kg to L-DOPA ($F(5,30) = 6.596$, $P < 0.001$, one-way RM ANOVA, Figure 1E). Thus, duration of ON-time without dyskinesia was 55 ± 16 min in the L-DOPA/ L-745,870 1 mg/kg treatment, compared to 15 ± 7 min in the L-DOPA/ vehicle treatment (267% increase, $P < 0.01$, Tukey's *post hoc* test), 15 ± 9 min in the L-DOPA/ L-745,870 0.01 mg/kg treatment (267% increase, $P < 0.01$, Tukey's *post hoc* test), 17 ± 10 min in the L-DOPA/ L-745,870 0.1 mg/kg treatment (230% increase, $P < 0.01$, Tukey's *post hoc* test) and

27 ± 7 min in the L-DOPA/ L-745,870 0.3 mg/kg treatment (106% increase, $P > 0.05$, Tukey's *post hoc* test).

L-745,870 does not influence L-DOPA anti-parkinsonian efficacy

Motor activity counts, parkinsonian scores and duration of ON-time were not significantly altered by the addition of L-745,870 to L-DOPA, when compared to L-DOPA/ vehicle treatment, indicating that L-745,870 did not impair the anti-parkinsonian action of L-DOPA.

Motor activity

Over the 360 min observation period, there was a significant effect of time, treatment and an interaction between the two variables on the motor activity of the macaques ($F_{\text{time}}(5,180) = 10.30$, $P < 0.001$, $F_{\text{treatment}}(6,180) = 29.03$, $P < 0.001$, and $F_{\text{interaction}}(30,180) = 5.179$, $P < 0.001$, two-way RM ANOVA, Figure 2A). There was a significant increase in motor activity counts following L-DOPA administration (in combination with vehicle or L-745,870), when compared to vehicle/ vehicle and vehicle/ L-745,870 treatments ($P < 0.05$ from 0-60 min and $P < 0.001$ from 120-180 min, Bonferroni's *post hoc* test). At no time during the observation period did L-745,870 have any effect on L-DOPA- or vehicle-induced motor activity ($P > 0.05$, Bonferroni's *post hoc* test). Accordingly, L-DOPA (in combination with vehicle or L-745,870) significantly increased total motor activity over the 360 min observation period, when compared to vehicle/ vehicle or vehicle/ L-745,870 treatments ($F(5,30) = 22.05$, $P < 0.001$, one-way RM ANOVA, $P < 0.001$, Tukey's *post hoc* test Figure 2B), while L-745,870 had no effect on L-DOPA- or vehicle-induced motor activity of the whole 360 min experiment ($P > 0.05$, Tukey's *post hoc* test).

Parkinsonian disability

Over the 360 min observation period, there was a significant effect of treatment and an interaction between time and treatment, but no effect of time alone on the degree of parkinsonism of the macaques ($F_{\text{time}}(5,210) = 0.0$, $P > 0.05$, $F_{\text{treatment}}(6,210) = 11.61$, $P < 0.001$, and $F_{\text{interaction}}(30,210) = 2.867$, $P < 0.001$, two-way ANOVA following ranking of data, Figure 3A). L-DOPA, whether combined with vehicle or L-745,870, significantly alleviated the severity of parkinsonism from 0-180 min following drug administration ($P < 0.05$ for all, Bonferroni's *post hoc* test). The addition of L-745,870 to vehicle or L-DOPA did not result in a worsening of parkinsonism ($P > 0.05$, Bonferroni's *post hoc* test).

ON-time duration

L-DOPA, in combination with vehicle or L-745,870 significantly extended duration of ON-time when compared to vehicle/ vehicle and vehicle/ L-745,870 treatments ($F(5,30) = 60.09$, $P < 0.001$, one-way RM ANOVA, $P < 0.001$, Tukey's *post hoc* test Figure 3B). The addition of L-745,870 to L-DOPA did not result in a reduction of ON-time duration ($P > 0.05$, Tukey's *post hoc* test); for instance, ON-time duration was 170 ± 15 min in the L-DOPA/ vehicle treatment compared to 186 ± 22 min following the L-DOPA/ L-745,870 1 mg/kg treatment ($P > 0.05$, Tukey's *post hoc* test).

Pharmacokinetic profile of L-745,870 in the MPTP-lesioned macaque

In the MPTP-lesioned macaque, L-745,870 was detectable in the plasma as early as 15 min after oral administration of the 1 mg/kg dose and was still above the detection threshold 24 h after administration. No L-745,870 was detectable in the pre-treatment sample.

PK parameters are illustrated in Figure 4 and detailed in Table 1. C_{max} reached after oral administration of L-745,870 1 mg/kg was 181.3 ± 5.3 nM and t_{max} was 6.0 ± 0.0 h. L-

745,870, $t_{1/2}$ was 4.2 ± 1.0 h and clearance rate was 0.072 ± 0.008 l/min. Bioavailability of L-745,870 was $42.0 \pm 11.5\%$. The shape of the curve following a single i.v. administration was indicative of first order kinetic metabolism at therapeutically-relevant plasma concentration (Figure 4B). L-DOPA had no effect on L-745,870 plasma PK time course (Figure 4C).

L-745,870 levels in the plasma, cerebrospinal fluid and brain of the MPTP-lesioned macaque at peak behavioural effect

As presented in Table 2, 90 min after oral administration, plasma L-745,870 levels were 193.9 ± 55.0 nmol/ml, while CSF levels were 12.7 ± 5.6 nmol/ml, which represents a 6% CSF/ plasma ratio. L-745,870 levels were 650 ± 263 ng/g wet tissue in the primary motor cortex and 560 ± 253 ng/g wet tissue in the putamen ($t = 0.2476$ df = 4, $P > 0.05$, one-tailed Student's *t* test).

Discussion

This study has demonstrated that oral administration of acute challenges of L-745,870 (1 mg/kg) in combination with L-DOPA significantly alleviates dyskinesia. Whether the anti-dyskinetic effect of L-745,870 would be maintained in the context of chronic administration of the drug remains unknown and further studies are needed to investigate the effects of long-term administration of L-745,870 on dyskinesia severity. The magnitude of the anti-dyskinetic effect achieved here with L-745,870 compares advantageously with the magnitude of the anti-dyskinetic effect we previously achieved with famotidine (Johnston et al., 2010c) and fipamezole (Johnston et al., 2010b). This reduction in dyskinesia severity was accompanied by a reduction in bad quality ON-time and an extension in good quality ON-time duration. Importantly, the anti-dyskinetic efficacy of L-745,870 was achieved without

compromising L-DOPA anti-parkinsonian benefit and at brain levels at which L-745,870 is selective for D₄ receptors, thereby identifying D₄ receptors as a promising target for dyskinesia. Moreover, effective plasma levels of L-745,870 obtained in our study are in the range of those that were demonstrated to be well-tolerated in human trials.

The anti-dyskinetic effect of L-745,870 is observed at doses at which it is known to act as a selective antagonist of dopamine D₄ receptors

L-745,870 is a potent and selective D₄ receptor antagonist with an inhibition constant (K_i) of 0.43 nM (Kulagowski et al., 1996; Patel et al., 1997; Stewart et al., 2004; Nakane et al., 2005). L-745,870 exhibits nearly 100-fold selectivity over its next target, alpha-2 adrenoreceptors (K_i of 33-49 nM) (Patel et al., 1997; Nakane et al., 2005).

The pharmacokinetics of L-745,870 had not been characterised previously in the cynomolgus macaque and had never been assessed in any animal model of parkinsonism. In comparison to the rhesus macaque (Patel et al., 1997), in cynomolgus macaques, L-745,870 has higher bioavailability but comparable t_{1/2}. However, the drug t_{1/2} is longer in human (15 h at steady state) (Bristow et al., 1997b). To our knowledge, most of the PK parameters reported in Figure 4, Table 1 and Table 2 have never been evaluated in the NHP and our study is the first to determine brain and CSF levels of L-745,870 in the cynomolgus macaque.

Plasma levels reached in our study are in accordance to those obtained in a human Phase IIa study (Kramer et al., 1997), in which plasma levels of L-745,870 were 12 ± 7 ng/ml, which corresponds to 36.7 ± 21.4 nmol/ml. Importantly, in that study, plasma was collected prior to medication intake, which corresponds to 1.6 drug half-lives. In our study, administration of L-745,870 1 mg/kg led to plasma levels slightly under 50 nmol/ml after 1.6 half-lives (approximately 7 h, Figure 4A). Thus, the effective dose in our study led to plasma concentrations similar to those that were demonstrated to be safe and well-tolerated by

human subjects. Moreover, at such plasma concentrations in human, L-745,870 remains highly selective for dopamine D₄ receptors and antagonises approximately 90% of D₄ receptors (Bristow et al., 1997b; Kramer et al., 1997).

Accordingly, we describe that the anti-dyskinetic actions of L-745,870 were seen at doses where brain levels were 650 ng/g tissue in the primary motor cortex and 560 ng/g tissue in the putamen, corresponding to concentrations of 397.8 nM in the motor cortex and 342.5 nM in the putamen. It has previously been demonstrated that only 2-2.5% of L-745,870 present within the brain is available for biological activity (Patel et al., 1997). Thus, with doses of L-745,870 providing anti-dyskinetic benefit, active brain levels would be 6-10 nM. Given the affinity of L-745,870 for D₄ receptors and selectivity described above, this would represent a concentration at which D₄ receptors were antagonised by > 95% while remaining highly selective for D₄ receptors, as 6-10 nM is well below the affinity of L-745,870 for its next target (Patel et al., 1997; Nakane et al., 2005). As such, it seems most likely that blockade of D₄ receptors is a promising target to alleviate L-DOPA-induced dyskinesia. However, further studies, with other dopamine D₄ receptor antagonists such as A-381,393 (*vide infra*), are required to validate D₄ receptor blockade as an effective means of alleviating dyskinesia.

L-745,870 does not impair L-DOPA anti-parkinsonian efficacy

The anti-dyskinetic effect of L-745,870 was achieved without compromising L-DOPA anti-parkinsonian action. Thus, at no time during the experiment did parkinsonism scores differ between L-DOPA/ L-745,870 and L-DOPA/ vehicle treatments. Additionally, the anti-dyskinetic effects of L-745,870 were not accompanied by a reduction in motor activity. Lastly, L-745,870 did not reduce ON-time duration, which was primarily defined by the absence of bradykinesia, based on the UK PD Society Brain Bank criteria for the

diagnosis of idiopathic PD (Hughes et al., 1992). However, as L-745,870 was not tested at doses above 1 mg/kg, it cannot be ruled out that higher doses of the compound might impair L-DOPA anti-parkinsonian action, though an interaction with targets other than D₄ receptors might then mediate such action. Accordingly, considering the argument put forth in the previous section, selective and virtually complete blockade of D₄ receptors does not seem to interfere with L-DOPA anti-parkinsonian efficacy.

Dopamine D₄ receptors and basal ganglia neurotransmission

While the current study validates the D₄ receptor as a therapeutic target for L-DOPA-induced dyskinesia, the mechanism(s) whereby antagonising D₄ receptors alleviates dyskinesia has yet to be determined. Because they exert similar effects on intracellular signalling, D₂, D₃ and D₄ receptors are members of the D₂-like receptor family (Missale et al., 1998). D₄ receptors are present within the striatum (Mauger et al., 1998; Rivera et al., 2002), in which they are located on medium-spiny neurons (Rivera et al., 2002). However, it remains unknown whether D₄ receptors are preferentially expressed on striatofugal neurons of the direct or indirect pathway. D₄ receptors are also found on large neurons of the globus pallidus (GP) (Ariano et al., 1997; Mauger et al., 1998). D₄ receptors are thus well localised to modulate dopaminergic nigrostriatal and nigropallidal transmissions. Moreover, D₄ receptor binding levels increase in the rat striatum following 6-OHDA lesion (Zhang et al., 2001), thereby providing an obvious site for potential enhancement of D₄ signalling as a mechanism underlying dyskinesia expression. According to the classic model of basal ganglia function (DeLong and Wichmann, 2007), antagonising striatal D₄ receptors along the indirect pathway would result, like antagonising D₂ receptors, in a reduction of dyskinesia, though at the expense of worsening the parkinsonian phenotype (Klawans and Weiner, 1974).

D₄ receptors are also encountered in the subthalamic nucleus (STN) (Flores et al., 1999). Stimulating D₄ receptors within the STN leads to a reduction of gamma-aminobutyric acid (GABA) levels, a phenomenon reversed by L-745,870 (Floran et al., 2004). According to the classic model of basal ganglia organisation (DeLong and Wichmann, 2007), reversing a reduction in GABA transmission within the STN would reduce dyskinesia, but would also compromise L-DOPA anti-parkinsonian action. On the other hand, antagonising striatal D₄ receptors along the direct pathway would exert an anti-parkinsonian effect (DeLong and Wichmann, 2007). D₄ receptors might thus be localised in such a way that it is possible to achieve balance between an anti-dyskinetic effect mediated by the indirect pathway and an anti-parkinsonian effect mediated by the direct pathway.

The preservation of the anti-parkinsonian action of L-DOPA demonstrated in the current study might also be explained by a direct effect of L-745,870 on the output structures of the basal ganglia. Thus, there is an important dopaminergic innervation of the GP pars interna (GPi) in primates (Parent et al., 1995) and these dopaminergic afferents are relatively spared following MPTP administration (Parent et al., 1990). Although less profuse, the substantia nigra pars reticulata (SNr) also receives dopaminergic input from the substantia nigra pars compacta (SNc) (Isaacs and Jacobowitz, 1994). Activation of D₄ receptors within the SNr results in a reduction of GP pars externa (GPe)-SNr GABA release, a phenomenon reversed by L-745,870 (Acosta-Garcia et al., 2009). Although such a phenomenon has not been demonstrated for the GPe-GPi projections, since some GPe-SNr fibres emit collaterals to the GPi (Sato et al., 2000), it is possible that L-745,870 exerts a similar effect on GPe-GPi GABAergic fibres, which would support an anti-parkinsonian benefit.

Another possible site mediating the anti-dyskinetic action of D₄ antagonists might be within the cortex. Thus, D₄ receptors modulate α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptor-mediated

currents in the frontal cortex (Rondou et al., 2010; Yuen et al., 2010). As such, antagonising D₄ receptors might decrease cortical excitability which, according to the classic model of basal ganglia organisation, would alleviate dyskinesia (DeLong and Wichmann, 2007).

L-745,870 as a development candidate in Parkinson's disease

L-745,870 appears to have several advantages over other drugs known to modulate D₄ receptors. For instance, clozapine binds to D₄ receptors with high affinity, but is not selective for this type of receptor (Huot et al., 2011) and, importantly, a 1-2% risk of agranulocytosis limits its use (Alvir et al., 1993). NGD 94-1 is a selective D₄ antagonist that exhibits 50-fold selectivity over 5-HT_{1A} receptors (Tallman et al., 1997), but its development does not seem to have been pursued. A-381,393 (Cowart et al., 2004; Nakane et al., 2005) is a selective D₄ receptor antagonist that exhibits over 2,000-fold selectivity over its next target. Although A-381,393 is more selective for D₄ receptors than L-745,870, the molecule is not available commercially and, to our knowledge, has not been tested in primates or humans; as such, its *in vivo* profile, PK, safety and tolerability have not been characterised and the drug's development path to clinic is considerably less advanced than that of L-745,870.

L-745,870 was initially developed as an antipsychotic compound and was assessed through to a Phase IIa study in acutely psychotic schizophrenic patients (Kramer et al., 1997). Although L-745,870 was ineffective in psychosis, the compound was demonstrated to be safe and well-tolerated in human subjects (Kramer et al., 1997), and its pharmacokinetics and pharmacodynamics have been well-characterised (Bristow et al., 1997b). The fact that the medication can be administered once daily also makes it attractive, especially in the context of PD, where patients experience dyskinesia as a complication of treatment throughout the waking day.

We propose that the safety and pharmacokinetic characteristics of L-745,870 make it an ideal candidate for rapid transition to clinical development. As such, the anti-dyskinetic efficacy of L-745,870 might be considered in the context of Phase II clinical trials in PD. Furthermore, as L-745,870 has already undergone significant clinical development, and has an excellent PK and safety profile for a therapeutic candidate, it could be advanced rapidly to such Phase IIa clinical studies for dyskinesia in PD. The translation of these findings would be facilitated by the therapeutically-relevant target levels for plasma and CSF exposure levels that we provide herein.

Authorship contributions

Participated in research design: Huot, Johnston, Koprach, Aman, Fox, Brotchie

Conducted experiments: Huot, Johnston, Koprach, Aman

Contributed new reagents or analytic tools: n/a

Performed data analysis: Huot, Aman, Brotchie

Wrote or contributed to the writing of the manuscript: Huot, Johnston, Koprach, Aman, Fox,
Brotchie

References

- Acosta-Garcia J, Hernandez-Chan N, Paz-Bermudez F, Sierra A, Erij D, Aceves J and Floran B (2009) D4 and D1 dopamine receptors modulate [3H] GABA release in the substantia nigra pars reticulata of the rat. *Neuropharmacology* **57**:725-730.
- Alvir JM, Lieberman JA, Safferman AZ, Schwimmer JL and Schaaf JA (1993) Clozapine-induced agranulocytosis. Incidence and risk factors in the United States. *N Engl J Med* **329**:162-167.
- Ariano MA, Wang J, Noblett KL, Larson ER and Sibley DR (1997) Cellular distribution of the rat D4 dopamine receptor protein in the CNS using anti-receptor antisera. *Brain Res* **752**:26-34.
- Bara-Jimenez W, Bibbiani F, Morris MJ, Dimitrova T, Sherzai A, Mouradian MM and Chase TN (2005) Effects of serotonin 5-HT1A agonist in advanced Parkinson's disease. *Mov Disord* **20**:932-936.
- Bartoszyk GD, Van Amsterdam C, Greiner HE, Rautenberg W, Russ H and Seyfried CA (2004) Sarizotan, a serotonin 5-HT1A receptor agonist and dopamine receptor ligand. 1. Neurochemical profile. *J Neural Transm* **111**:113-126.
- Bristow LJ, Collinson N, Cook GP, Curtis N, Freedman SB, Kulagowski JJ, Leeson PD, Patel S, Ragan CI, Ridgill M, Saywell KL and Tricklebank MD (1997a) L-745,870, a subtype selective dopamine D4 receptor antagonist, does not exhibit a neuroleptic-like profile in rodent behavioral tests. *J Pharmacol Exp Ther* **283**:1256-1263.
- Bristow LJ, Kramer MS, Kulagowski J, Patel S, Ragan CI and Seabrook GR (1997b) Schizophrenia and L-745,870, a novel dopamine D4 receptor antagonist. *Trends Pharmacol Sci* **18**:186-188.
- Cowart M, Latshaw SP, Bhatia P, Daanen JF, Rohde J, Nelson SL, Patel M, Kolasa T, Nakane M, Uchic ME, Miller LN, Terranova MA, Chang R, Donnelly-Roberts DL,

- Namovic MT, Hollingsworth PR, Martino BR, Lynch JJ, 3rd, Sullivan JP, Hsieh GC, Moreland RB, Brioni JD and Stewart AO (2004) Discovery of 2-(4-pyridin-2-ylpiperazin-1-ylmethyl)-1H-benzimidazole (ABT-724), a dopaminergic agent with a novel mode of action for the potential treatment of erectile dysfunction. *J Med Chem* **47**:3853-3864.
- DeLong MR (1990) Primate models of movement disorders of basal ganglia origin. *Trends Neurosci* **13**:281-285.
- DeLong MR and Wichmann T (2007) Circuits and circuit disorders of the basal ganglia. *Arch Neurol* **64**:20-24.
- Dupre KB, Eskow KL, Barnum CJ and Bishop C (2008) Striatal 5-HT_{1A} receptor stimulation reduces D1 receptor-induced dyskinesia and improves movement in the hemiparkinsonian rat. *Neuropharmacology* **55**:1321-1328.
- Ehringer H and Hornykiewicz O (1960) [Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system.]. *Klin Wochenschr* **38**:1236-1239.
- Fabbrini G, Brotchie JM, Grandas F, Nomoto M and Goetz CG (2007) Levodopa-induced dyskinesias. *Mov Disord* **22**:1379-1389; quiz 1523.
- Fahn S (2008) The history of dopamine and levodopa in the treatment of Parkinson's disease. *Mov Disord* **23 Suppl 3**:S497-508.
- Floran B, Floran L, Eriq D and Aceves J (2004) Dopamine D₄ receptors inhibit depolarization-induced [3H]GABA release in the rat subthalamic nucleus. *Eur J Pharmacol* **498**:97-102.
- Flores G, Liang JJ, Sierra A, Martinez-Fong D, Quirion R, Aceves J and Srivastava LK (1999) Expression of dopamine receptors in the subthalamic nucleus of the rat:

characterization using reverse transcriptase-polymerase chain reaction and autoradiography. *Neuroscience* **91**:549-556.

Goetz CG, Laska E, Hicking C, Damier P, Muller T, Nutt J, Warren Olanow C, Rascol O and Russ H (2008) Placebo influences on dyskinesia in Parkinson's disease. *Mov Disord* **23**:700-707.

Gold SJ, Hoang CV, Potts BW, Porras G, Pioli E, Kim KW, Nadjar A, Qin C, LaHoste GJ, Li Q, Bioulac BH, Waugh JL, Gurevich E, Neve RL and Bezard E (2007) RGS9-2 negatively modulates L-3,4-dihydroxyphenylalanine-induced dyskinesia in experimental Parkinson's disease. *J Neurosci* **27**:14338-14348.

Gregoire L, Samadi P, Graham J, Bedard PJ, Bartoszyk GD and Di Paolo T (2009) Low doses of sarizotan reduce dyskinesias and maintain antiparkinsonian efficacy of L-Dopa in parkinsonian monkeys. *Parkinsonism Relat Disord* **15**:445-452.

Guigoni C, Doudnikoff E, Li Q, Bloch B and Bezard E (2007) Altered D(1) dopamine receptor trafficking in parkinsonian and dyskinetic non-human primates. *Neurobiol Dis* **26**:452-463.

Hadj Tahar A, Belanger N, Bangassoro E, Gregoire L and Bedard PJ (2000) Antidyskinetic effect of JL-18, a clozapine analog, in parkinsonian monkeys. *Eur J Pharmacol* **399**:183-186.

Hassler R (1938) Zur pathologie der paralysis agitans und des postenzephalitischen parkinsonismus. *J Psychol Neurol (Lpz)* **48**:387-476.

Hely MA, Morris JG, Reid WG and Trafficante R (2005) Sydney Multicenter Study of Parkinson's disease: non-L-dopa-responsive problems dominate at 15 years. *Mov Disord* **20**:190-199.

- Hughes AJ, Daniel SE, Kilford L and Lees AJ (1992) Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry* **55**:181-184.
- Huot P, Fox SH and Brotchie JM (2011) The serotonergic system in Parkinson's disease. *Prog Neurobiol* **95**:163-212.
- Isaacs KR and Jacobowitz DM (1994) Mapping of the colocalization of calretinin and tyrosine hydroxylase in the rat substantia nigra and ventral tegmental area. *Exp Brain Res* **99**:34-42.
- Jenner P (2008) Molecular mechanisms of L-DOPA-induced dyskinesia. *Nat Rev Neurosci* **9**:665-677.
- Johnston TH, Fox SH, McIlldowie MJ, Piggott MJ and Brotchie JM (2010a) Reduction of L-DOPA-induced dyskinesia by the selective metabotropic glutamate receptor 5 antagonist 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned macaque model of Parkinson's disease. *J Pharmacol Exp Ther* **333**:865-873.
- Johnston TH, Fox SH, Piggott MJ, Savola JM and Brotchie JM (2010b) The alpha adrenergic antagonist fipamezole improves quality of levodopa action in Parkinsonian primates. *Mov Disord* **25**:2084-2093.
- Johnston TH, van der Meij A, Brotchie JM and Fox SH (2010c) Effect of histamine H2 receptor antagonism on levodopa-induced dyskinesia in the MPTP-macaque model of Parkinson's disease. *Mov Disord* **25**:1379-1390.
- Klawans HL, Jr. and Weiner WJ (1974) Attempted use of haloperidol in the treatment of L-dopa induced dyskinesias. *J Neurol Neurosurg Psychiatry* **37**:427-430.

- Kramer MS, Last B, Getson A and Reines SA (1997) The effects of a selective D4 dopamine receptor antagonist (L-745,870) in acutely psychotic inpatients with schizophrenia. D4 Dopamine Antagonist Group. *Arch Gen Psychiatry* **54**:567-572.
- Kulagowski JJ, Broughton HB, Curtis NR, Mawer IM, Ridgill MP, Baker R, Emms F, Freedman SB, Marwood R, Patel S, Patel S, Ragan I and Leeson PD (1996) 3-[[4-(4-chlorophenyl)piperazin-1-yl]-methyl]-1H-pyrrolo[2,3-b]pyridine: an antagonist with high affinity and selectivity for the human dopamine D4 receptor. *J Med Chem* **39**.
- Liegeois JF, Bruhwyler J, Damas J, Rogister F, Masereel B, Geczy J and Delarge J (1995) Modulation of the clozapine structure increases its selectivity for the dopamine D4 receptor. *Eur J Pharmacol* **273**:R1-3.
- Marin C, Aguilar E, Rodriguez-Oroz MC, Bartoszyk GD and Obeso JA (2009) Local administration of sarizotan into the subthalamic nucleus attenuates levodopa-induced dyskinesias in 6-OHDA-lesioned rats. *Psychopharmacology (Berl)* **204**:241-250.
- Mauger C, Sivan B, Brockhaus M, Fuchs S, Civelli O and Monsma F, Jr. (1998) Development and characterization of antibodies directed against the mouse D4 dopamine receptor. *Eur J Neurosci* **10**:529-537.
- Missale C, Nash SR, Robinson SW, Jaber M and Caron MG (1998) Dopamine receptors: from structure to function. *Physiol Rev* **78**:189-225.
- Nakane M, Cowart MD, Hsieh GC, Miller L, Uchic ME, Chang R, Terranova MA, Donnelly-Roberts DL, Namovic MT, Miller TR, Wetter JM, Marsh K, Stewart AO, Brioni JD and Moreland RB (2005) 2-[4-(3,4-Dimethylphenyl)piperazin-1-ylmethyl]-1H-benzimidazole (A-381393), a selective dopamine D4 receptor antagonist. *Neuropharmacology* **49**:112-121.

- NIH (1996):N. Institute of Laboratory Animal Resources (U.S.). Committee on Care and Use of Laboratory Animals. (U.S. Dept. of Health and Human Services, Public Health Service, Bethesda, Md., 1996.
- Olanow CW, Damier P, Goetz CG, Mueller T, Nutt J, Rascol O, Serbanescu A, Deckers F and Russ H (2004) Multicenter, open-label, trial of sarizotan in Parkinson disease patients with levodopa-induced dyskinesias (the SPLENDID Study). *Clin Neuropharmacol* **27**:58-62.
- Parent A, Cote PY and Lavoie B (1995) Chemical anatomy of primate basal ganglia. *Prog Neurobiol* **46**:131-197.
- Parent A, Lavoie B, Smith Y and Bedard P (1990) The dopaminergic nigropallidal projection in primates: distinct cellular origin and relative sparing in MPTP-treated monkeys. *Adv Neurol* **53**:111-116.
- Patel S, Freedman S, Chapman KL, Emms F, Fletcher AE, Knowles M, Marwood R, McAllister G, Myers J, Curtis N, Kulagowski JJ, Leeson PD, Ridgill M, Graham M, Matheson S, Rathbone D, Watt AP, Bristow LJ, Rupniak NM, Baskin E, Lynch JJ and Ragan CI (1997) Biological profile of L-745,870, a selective antagonist with high affinity for the dopamine D4 receptor. *J Pharmacol Exp Ther* **283**:636-647.
- Pecchevis M, Clarke CE, Vieregge P, Khoshnood B, Deschaseaux-Voinet C, Berdeaux G and Ziegler M (2005) Effects of dyskinesias in Parkinson's disease on quality of life and health-related costs: a prospective European study. *Eur J Neurol* **12**:956-963.
- Rabiner EA, Gunn RN, Wilkins MR, Sedman E and Grasby PM (2002) Evaluation of EMD 128 130 occupancy of the 5-HT1A and the D2 receptor: a human PET study with [11C]WAY-100635 and [11C]raclopride. *J Psychopharmacol* **16**:195-199.
- Rascol O, Brooks DJ, Melamed E, Oertel W, Poewe W, Stocchi F and Tolosa E (2005) Rasagiline as an adjunct to levodopa in patients with Parkinson's disease and motor

- fluctuations (LARGO, Lasting effect in Adjunct therapy with Rasagiline Given Once daily, study): a randomised, double-blind, parallel-group trial. *Lancet* **365**:947-954.
- Rivera A, Cuellar B, Giron FJ, Grandy DK, de la Calle A and Moratalla R (2002) Dopamine D4 receptors are heterogeneously distributed in the striosomes/matrix compartments of the striatum. *J Neurochem* **80**:219-229.
- Rondou P, Haegeman G and Van Craenenbroeck K (2010) The dopamine D4 receptor: biochemical and signalling properties. *Cell Mol Life Sci* **67**:1971-1986.
- Sato F, Lavallee P, Levesque M and Parent A (2000) Single-axon tracing study of neurons of the external segment of the globus pallidus in primate. *J Comp Neurol* **417**:17-31.
- Stewart AO, Cowart MD, Moreland RB, Latshaw SP, Matulenko MA, Bhatia PA, Wang X, Daanen JF, Nelson SL, Terranova MA, Namovic MT, Donnelly-Roberts DL, Miller LN, Nakane M, Sullivan JP and Brioni JD (2004) Dopamine D4 ligands and models of receptor activation: 2-(4-pyridin-2-ylpiperazin-1-ylmethyl)-1H-benzimidazole and related heteroarylmethylarylpiperazines exhibit a substituent effect responsible for additional efficacy tuning. *J Med Chem* **47**:2348-2355.
- Tallman JF, Primus RJ, Brodbeck R, Cornfield L, Meade R, Woodruff K, Ross P, Thurkauf A and Gallager DW (1997) I. NGD 94-1: identification of a novel, high-affinity antagonist at the human dopamine D4 receptor. *J Pharmacol Exp Ther* **282**:1011-1019.
- Visanji NP, Fox SH, Johnston T, Reyes G, Millan MJ and Brotchie JM (2009a) Dopamine D3 receptor stimulation underlies the development of L-DOPA-induced dyskinesia in animal models of Parkinson's disease. *Neurobiol Dis* **35**:184-192.
- Visanji NP, Fox SH, Johnston TH, Millan MJ and Brotchie JM (2009b) Alpha1-adrenoceptors mediate dihydroxyphenylalanine-induced activity in 1-methyl-4-

phenyl-1,2,3,6-tetrahydropyridine-lesioned macaques. *J Pharmacol Exp Ther* **328**:276-283.

Yuen EY, Zhong P and Yan Z (2010) Homeostatic regulation of glutamatergic transmission by dopamine D4 receptors. *Proc Natl Acad Sci U S A* **107**:22308-22313.

Zhang K, Tarazi FI and Baldessarini RJ (2001) Nigrostriatal dopaminergic denervation enhances dopamine D4 receptor binding in rat caudate-putamen. *Pharmacol Biochem Behav* **69**:111-116.

Footnotes

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There are no conflicts of interest. SHF has received consultancy fees from Merck, Merck Serono and Teva. JMB holds an equity position in Atuka Ltd. PH, THJ, JBK and JMB have received consultancy fees from Atuka Ltd and Atuka Inc. TH, JBK and JMB hold equity positions in Atuka Inc. AA has no financial disclosures.

* THJ and JBK contributed equally to the work

Figure legends

Figure 1: dyskinesia and quality of ON-time

- A. Time course of dyskinesia in macaques treated with vehicle or L-DOPA in combination with vehicle or L-745,870 (0.01, 0.1, 0.3 and 1 mg/kg). No dyskinesia were elicited by the vehicle/ vehicle and vehicle/ L-745,870 treatments. In contrast, L-DOPA, in combination with vehicle or L-745,870, elicited dyskinesia. However, the addition of L-745,870 1 mg/kg to L-DOPA significantly alleviated dyskinesia severity ($P < 0.01$ when compared to L-DOPA/ vehicle from 0-180 min, $P < 0.01$ when compared to L-DOPA/ L-745,870 0.01 mg/kg from 60-180 min, $P < 0.05$ when compared to L-DOPA/ L-745,870 0.1 mg/kg from 120-180 min).
- B. Duration of ON-time with dyskinesia in macaques treated with vehicle or L-DOPA in combination with vehicle or L-745,870 (0.01, 0.1, 0.3 and 1 mg/kg). Duration of ON-time with dyskinesia was significantly longer following L-DOPA administration, in combination with vehicle or L-745,870 when compared to either vehicle/ vehicle or vehicle/ L-745,870 treatments (both $P < 0.001$). The addition of L-745,870 to L-DOPA did not modify duration of ON-time with dyskinesia, regardless of the dose (all $P > 0.05$ when compared to L-DOPA/ vehicle).
- C. Duration of ON-time with disabling dyskinesia in macaques treated with vehicle or L-DOPA in combination with vehicle or L-745,870 (0.01, 0.1, 0.3 and 1 mg/kg). L-745,870 1 mg/kg significantly reduced duration of ON-time with disabling dyskinesia when compared to L-DOPA/ vehicle ($P < 0.001$), L-DOPA/ L-745,870 0.01 mg/kg ($P < 0.001$), L-DOPA/ L-745,870 0.1 mg/kg ($P < 0.05$) and L-DOPA/ L-745,870 0.3 mg/kg ($P < 0.05$) treatments.

- D. Duration of ON-time without disabling dyskinesia in macaques treated with vehicle or L-DOPA in combination with vehicle or L-745,870 (0.01, 0.1, 0.3 and 1 mg/kg). L-745,870 1 mg/kg significantly extended duration of ON-time without disabling dyskinesia when compared to L-DOPA/ vehicle ($P < 0.001$), L-DOPA/ L-745,870 0.01 mg/kg ($P < 0.001$), L-DOPA/ L-745,870 0.1 mg/kg ($P < 0.01$) and L-DOPA/ L-745,870 0.3 mg/kg ($P < 0.01$) treatments.
- E. Duration of ON-time without dyskinesia in macaques treated with vehicle or L-DOPA in combination with vehicle or L-745,870 (0.01, 0.1, 0.3 and 1 mg/kg). L-745,870 1 mg/kg significantly extended duration of ON-time without dyskinesia when compared to L-DOPA/ vehicle ($P < 0.01$), L-DOPA/ L-745,870 0.01 mg/kg ($P < 0.01$), and L-DOPA/ L-745,870 0.1 mg/kg ($P < 0.01$) treatments.

In A, each dot represents the median dyskinesia score for the preceding 60 min period; the maximal possible score (most severe disability) was 24 and on the y-axis, mild = 6, moderate = 12, marked = 18, severe = 24. In B, C, D and E, the bars represent the mean \pm SEM ON-time duration. The crosses in A represent time points for which there is statistical

Figure 2: motor activity

When combined to L-DOPA, L-745,870 did not alter motor activity, as reflected on the motor activity time course (A) or the motor activity counts for the 360 recording period (B). In both A and B, L-DOPA, in combination with vehicle or L-745,870, elicited significantly higher motor activity counts than vehicle/ vehicle or vehicle/ L-745,870 (both $P < 0.001$).

In A, each dot represents the mean motor activity counts for the preceding 60 min period, whereas in B, each bar represents the mean \pm SEM motor activity counts for the 360 min duration of the experiment.

*: $P < 0.05$ when compared to vehicle/ vehicle; **: $P < 0.01$ when compared to vehicle/ vehicle; ***: $P < 0.001$ when compared to vehicle/ vehicle; #: $P < 0.05$ when compared to vehicle/ L-745,870; ##: $P < 0.01$ when compared to vehicle/ L-745,870; ###: $P < 0.001$ when compared to vehicle/ L-745,870.

Figure 3: degree of parkinsonism and duration of L-DOPA anti-parkinsonian action

When combined to L-DOPA, L-745,870 had no effect on the degree of parkinsonism over the 360 min behavioural observation (A). Accordingly, L-745,870 did not alter duration of L-DOPA anti-parkinsonian action, ON-time (B). In both A and B, L-DOPA, in combination with either vehicle or L-745,870, significantly reversed parkinsonian disability ($P < 0.001$), as manifested by a reduction in parkinsonian score (A) and an extension of ON-time (B).

In A, each dot represents the median parkinsonian score for the preceding 60 min period; the maximal possible score (most severe disability) was 60 and on the y-axis, mild = 15, moderate = 30, marked = 45, severe = 60. In B, the bars represent the mean \pm SEM ON-time duration.

*: $P < 0.05$ when compared to vehicle/ vehicle; **: $P < 0.01$ when compared to vehicle/ vehicle; ***: $P < 0.001$ when compared to vehicle/ vehicle; #: $P < 0.05$ when compared to vehicle/ L-745,870; ##: $P < 0.01$ when compared to vehicle/ L-745,870; ###: $P < 0.001$ when compared to vehicle/ L-745,870; §: $P < 0.05$ when compared to L-DOPA/ vehicle.

Figure 4: pharmacokinetics of L-745,870 in the MPTP-lesioned macaque

- A. Time course of L-745,870 (vehicle, 0.1, 0.3 and 1.0 mg/kg) plasma levels following oral administration.
- B. Time course of L-745,870 (0.3 mg/kg) plasma levels following intravenous administration. The shape of the curve is indicative of a first order kinetic metabolism.

C. Time course of L-745,870 (0.3 mg/kg) plasma levels following oral administration in combination and in the absence of L-DOPA (30 mg/kg p.o.). L-DOPA administration had no effect on plasma L-745,870 levels or L-745,870 pharmacokinetic parameters (all $P > 0.05$).

AUC: area under curve; C_{\max} : maximal plasma concentration; t_{\max} : time to reach C_{\max} . Data are presented as the mean \pm SEM L-745,870 plasma levels.

Table 1: L-745,870 pharmacokinetic parameters in the MPTP-lesioned macaque

L-745,870	L-745,870 (mg/kg)			
	0.1 p.o.	0.3 p.o.	1.0 p.o.	0.3 i.v.
C_{max} (nmol/ml), 0-1h	3.4 ± 2.6	11.1 ± 9.4	97.8 ± 22.8	183.8 ± 13.8
C_{max} (nmol/ml), 0-2h	9.8 ± 3.4	27.5 ± 8.6	142.7 ± 12.3	183.8 ± 13.8
C_{max} (nmol/ml), 0-3h	10.1 ± 3.2	28.4 ± 7.7	181.3 ± 5.3	183.8 ± 13.8
C_{max} (nmol/ml), 0-6h	10.1 ± 3.2	28.6 ± 7.6	181.3 ± 5.3	183.8 ± 13.8
C_{max} (nmol/ml), 0-8h	10.1 ± 3.2	28.6 ± 7.6	181.3 ± 5.3	183.8 ± 13.8
C_{max} (nmol/ml), 0-24h	10.1 ± 3.2	28.6 ± 7.6	181.3 ± 5.3	183.8 ± 13.8
AUC ₀₋₁ (h.nmol/ml)	0.8 ± 0.6	3.0 ± 2.6	55.9 ± 18.8	135.2 ± 10.3
AUC ₀₋₂ (h.nmol/ml)	7.5 ± 3.6	22.4 ± 9.7	176.1 ± 36.2	236.1 ± 21.6
AUC ₀₋₃ (h.nmol/ml)	16.1 ± 5.9	48.8 ± 15.4	338.1 ± 42.3	307.1 ± 40.4
AUC ₀₋₆ (h.nmol/ml)	33.3 ± 9.9	112.4 ± 28.9	700.9 ± 15.4	431.6 ± 72.7
AUC ₀₋₈ (h.nmol/ml)	38.5 ± 11.0	142.0 ± 35.6	767.1 ± 11.9	477.4 ± 79.9
AUC ₀₋₂₄ (h.nmol/ml)	50.7 ± 6.1	266.9 ± 77.6	815.5 ± 51.9	648.3 ± 84.2
t_{max} (h)	2.3 ± 0.3	3.3 ± 1.3	3.0 ± 0.0	0.3 ± 0.1
k_{el} (h ⁻¹)				0.18 ± 0.04
$t_{1/2}$ (h)				4.24 ± 1.02
CL (l/h)				0.072 ± 0.008
bioavailability (%)		42.0 ± 11.5		

AUC: area under curve; CL: clearance; C_{max} : maximal plasma concentration; k_{el} : elimination rate constant; $t_{1/2}$: half-life; t_{max} : time to reach C_{max} . The AUC₀₋₂₄ values were used to calculate the CL. Values are presented as the mean ± SEM.

Table 2: L-745,870 levels in the brain and cerebrospinal fluid in the MPTP-lesioned cynomolgus macaque at peak behavioural effect

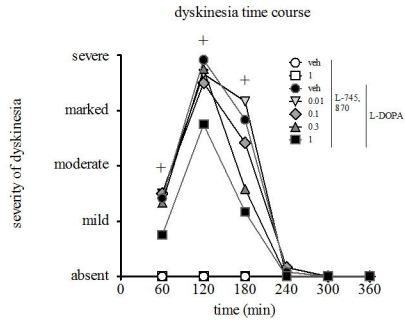
	L-745,870 (1 mg/kg p.o.)
plasma (nmol/ml)	193.9±55.0
CSF (nmol/ml)	12.7±5.6
motor cortex (ng/g)	650±263
putamen (ng/g)	560±253
CSF/plasma ratio	0.06±0.02

CSF: cerebrospinal fluid.

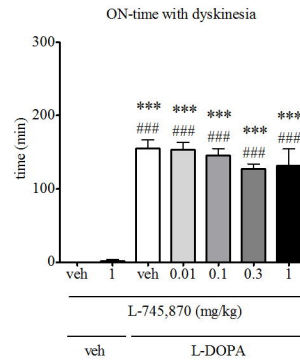
For the cortex and putamen, data are expressed as ng/g wet tissue.

Figure 1

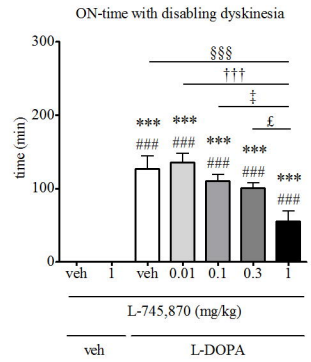
A



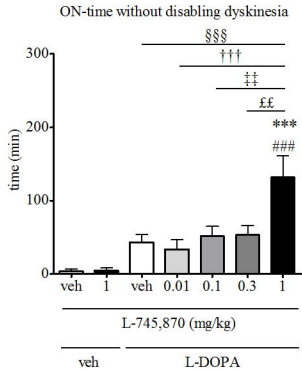
B



C



D



E

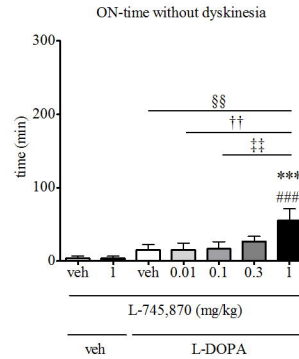
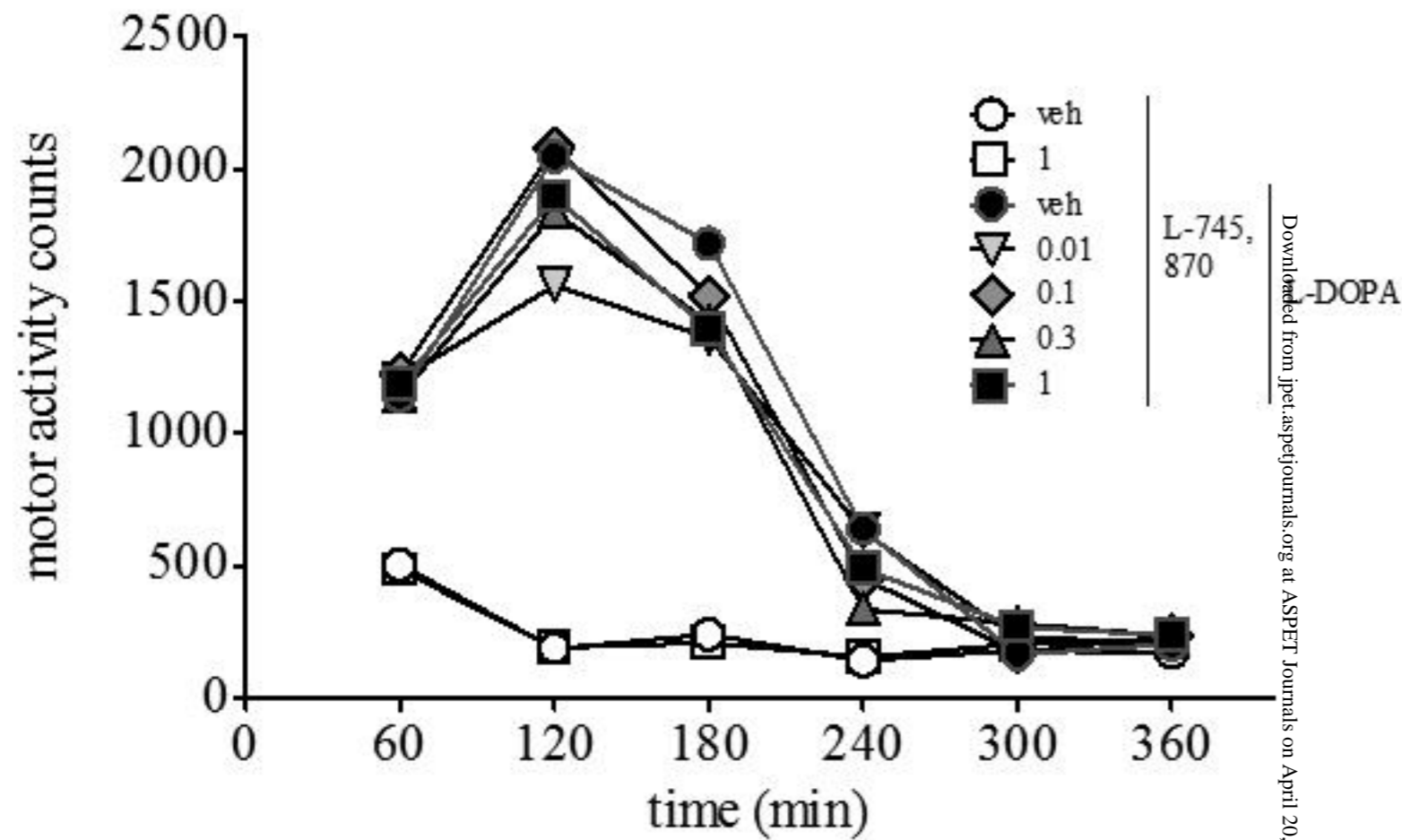


Figure 2

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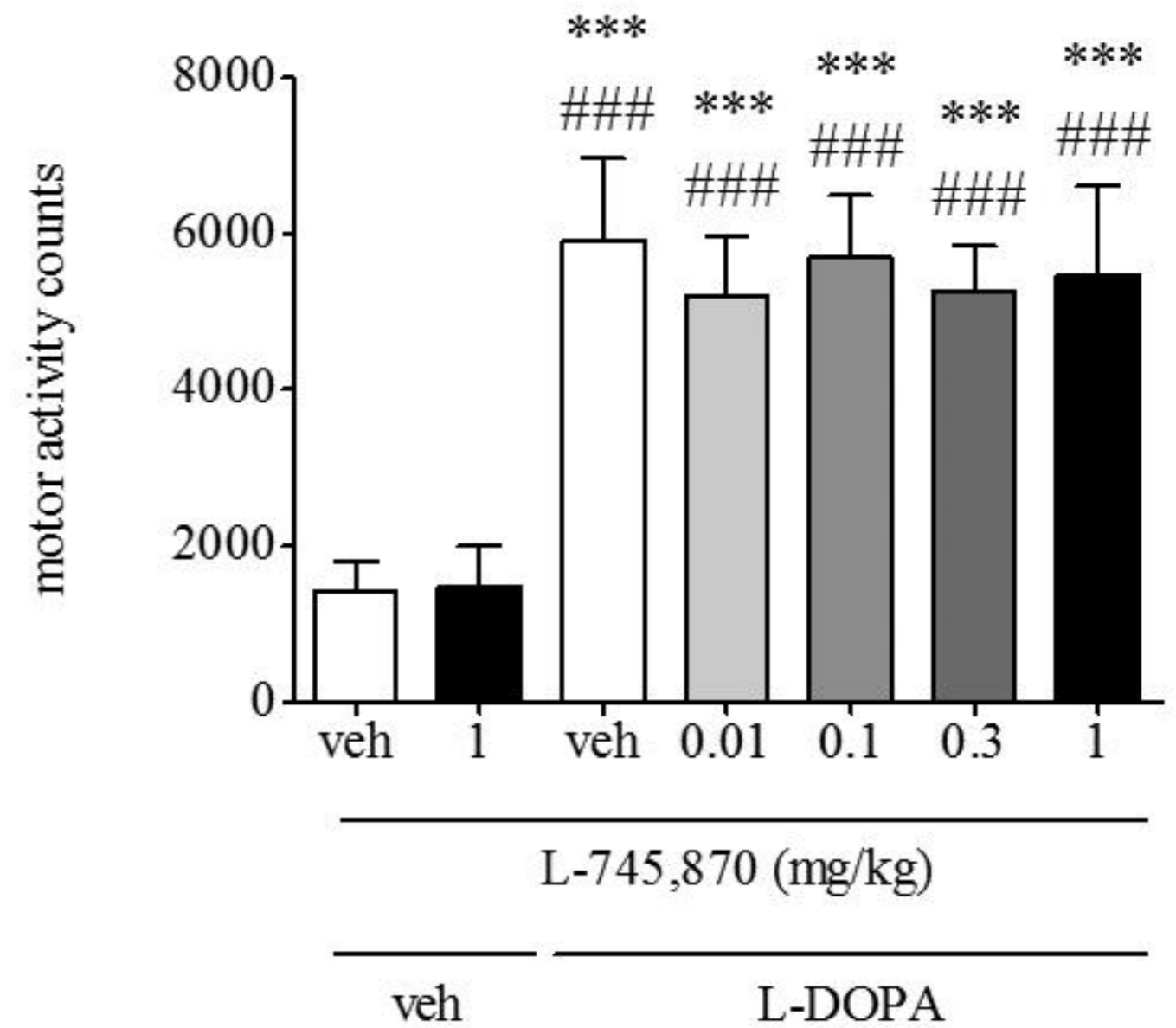
A

motor activity time course



B

motor activity 0 - 360 min



		veh/veh cf.					
L-745,870 (mg/kg)		60	120	180	240	300	360
vehicle	1	ns	ns	ns	ns	ns	ns
	vehicle	*,#	***,###	***,###	ns	ns	ns
	0.01	** ,###	***,###	***,###	ns	ns	ns
L-DOPA	0.1	** ,###	***,###	***,###	ns	ns	ns
	0.3	*,#	***,###	***,###	ns	ns	ns
	1	** ,###	***,###	***,###	ns	ns	ns

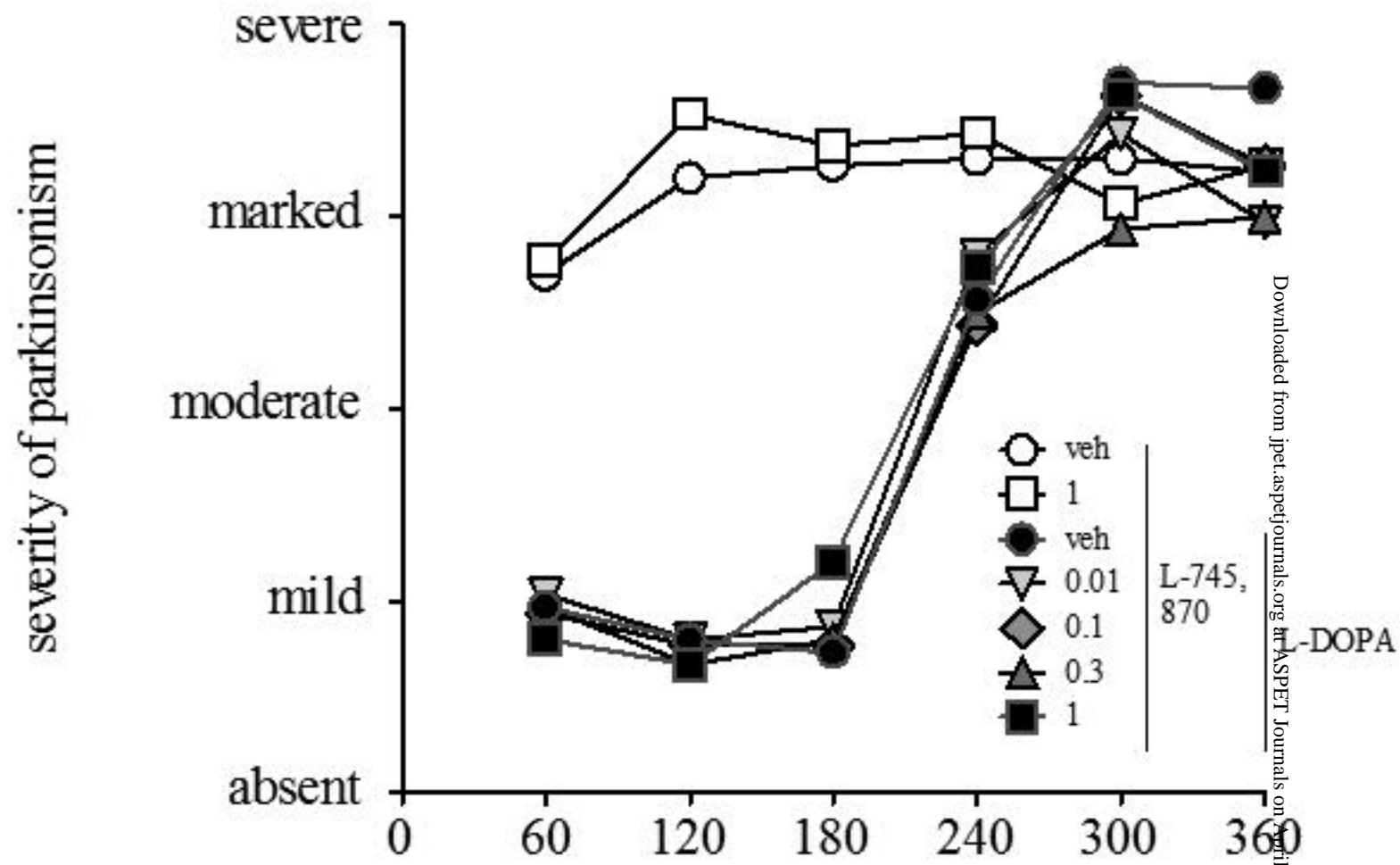
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Figure 3

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A

parkinsonism time course



		veh/veh cf.						
		L-745,870 (mg/kg)	60	120	180	240	300	360
vehicle	1		ns	ns	ns	ns	ns	ns
L-DOPA	vehicle		** , ##	** , ###	*** , ###	ns	ns	ns
	0.01		* , ##	#	** , ###	ns	ns	§
	0.1		*** , ###	** , ###	*** , ###	ns	ns	ns
	0.3		#	*** , ###	** , ###	ns	ns	ns
	1		*** , ###	** , ###	ns	ns	ns	§

B

ON-time

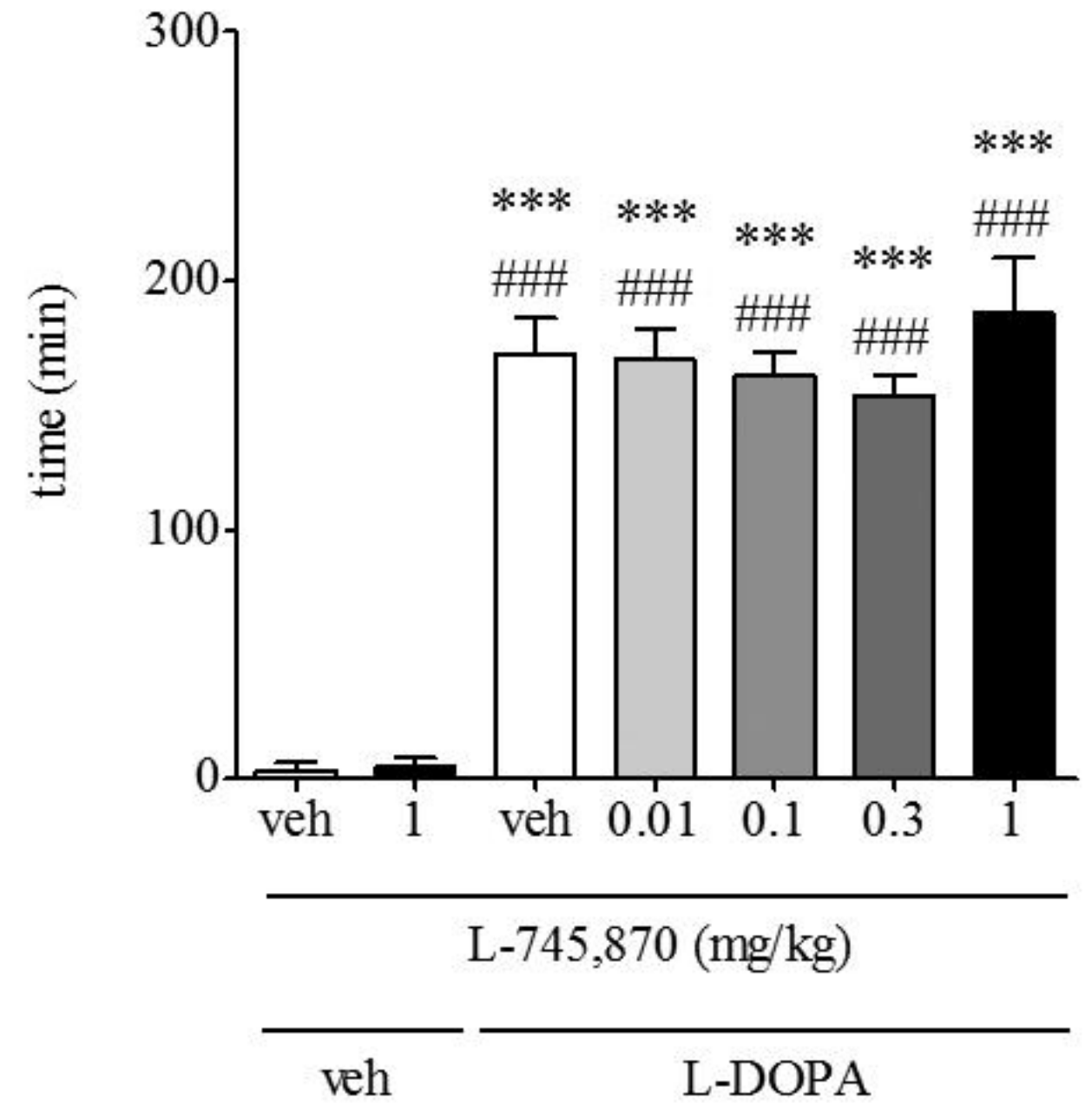
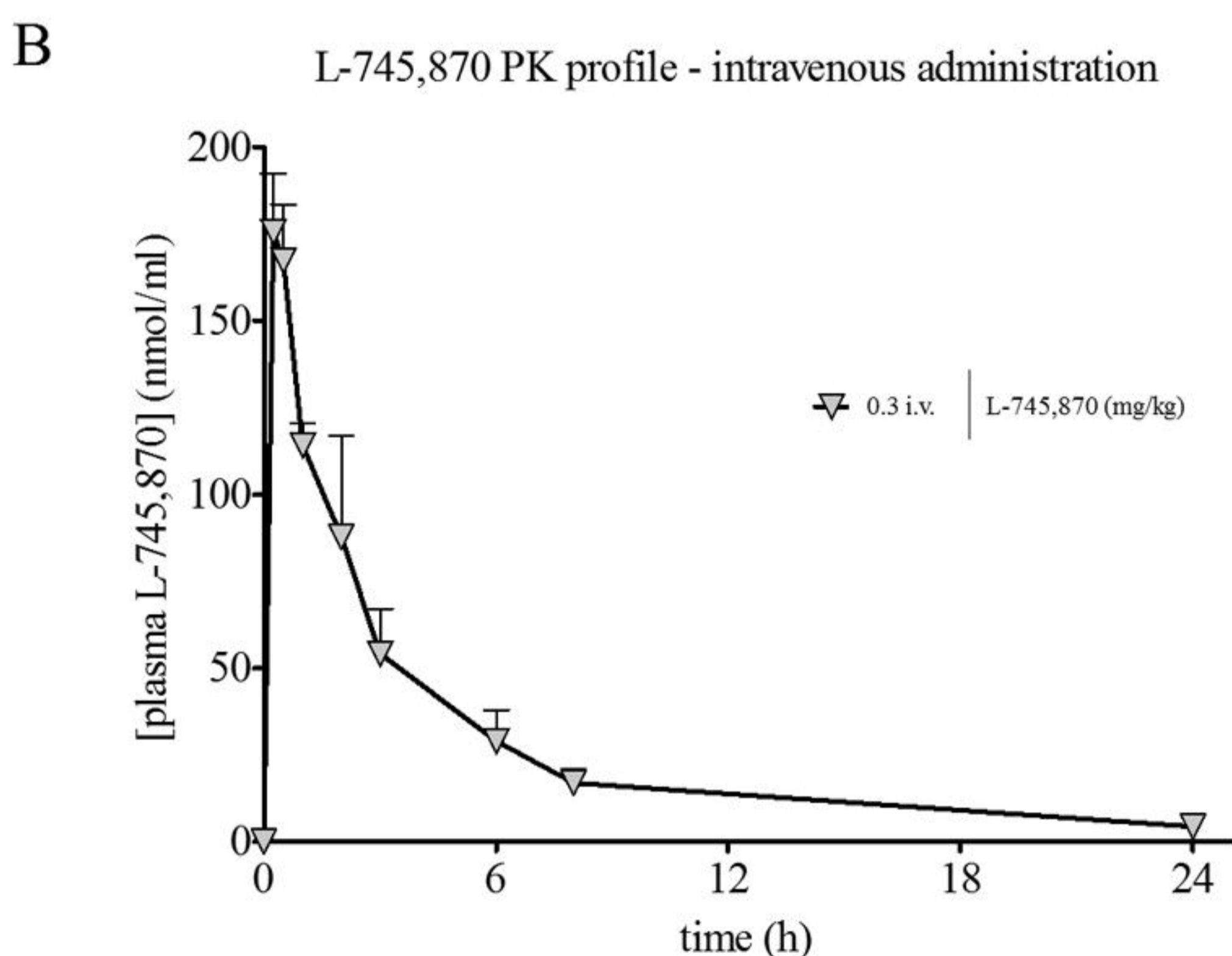
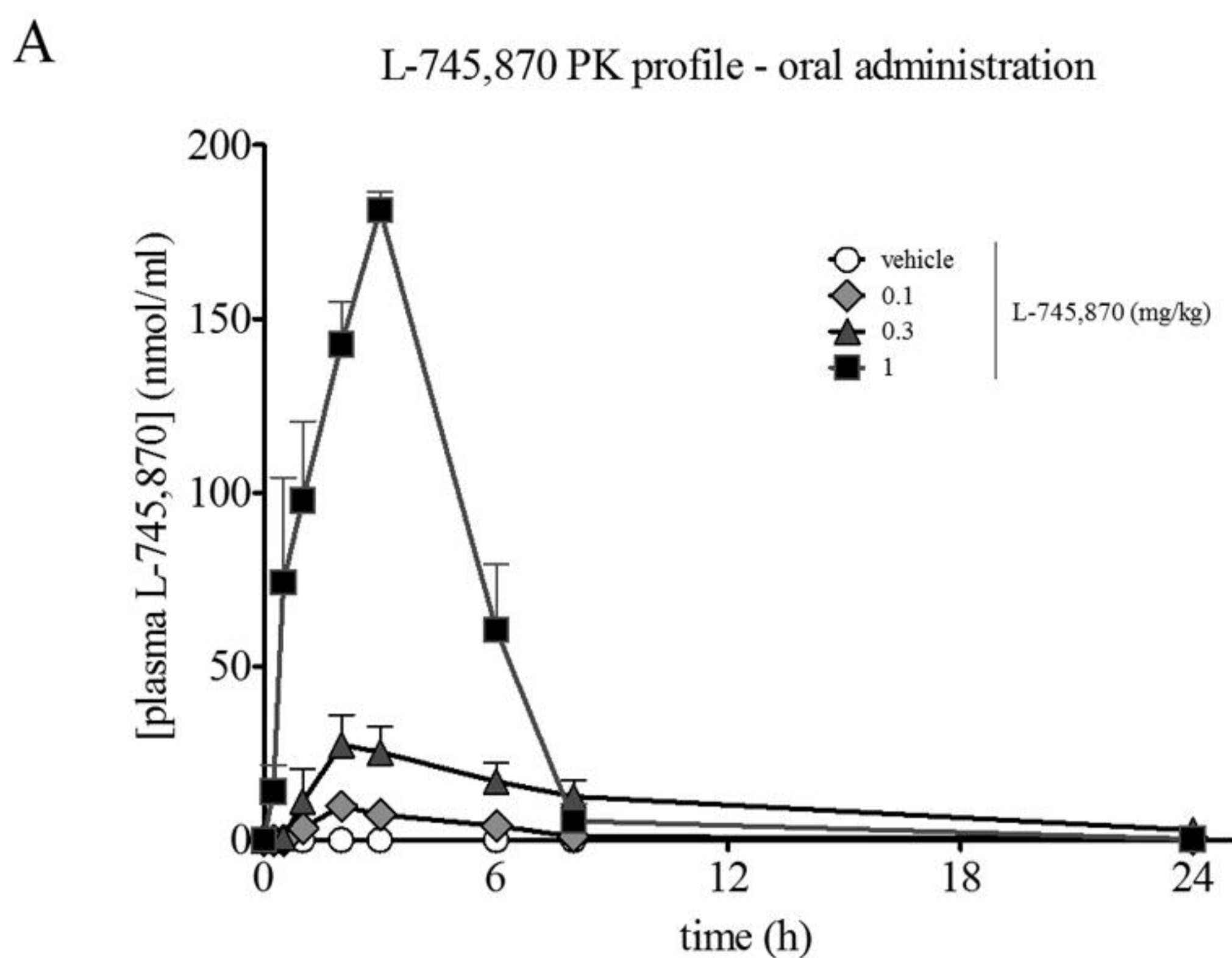
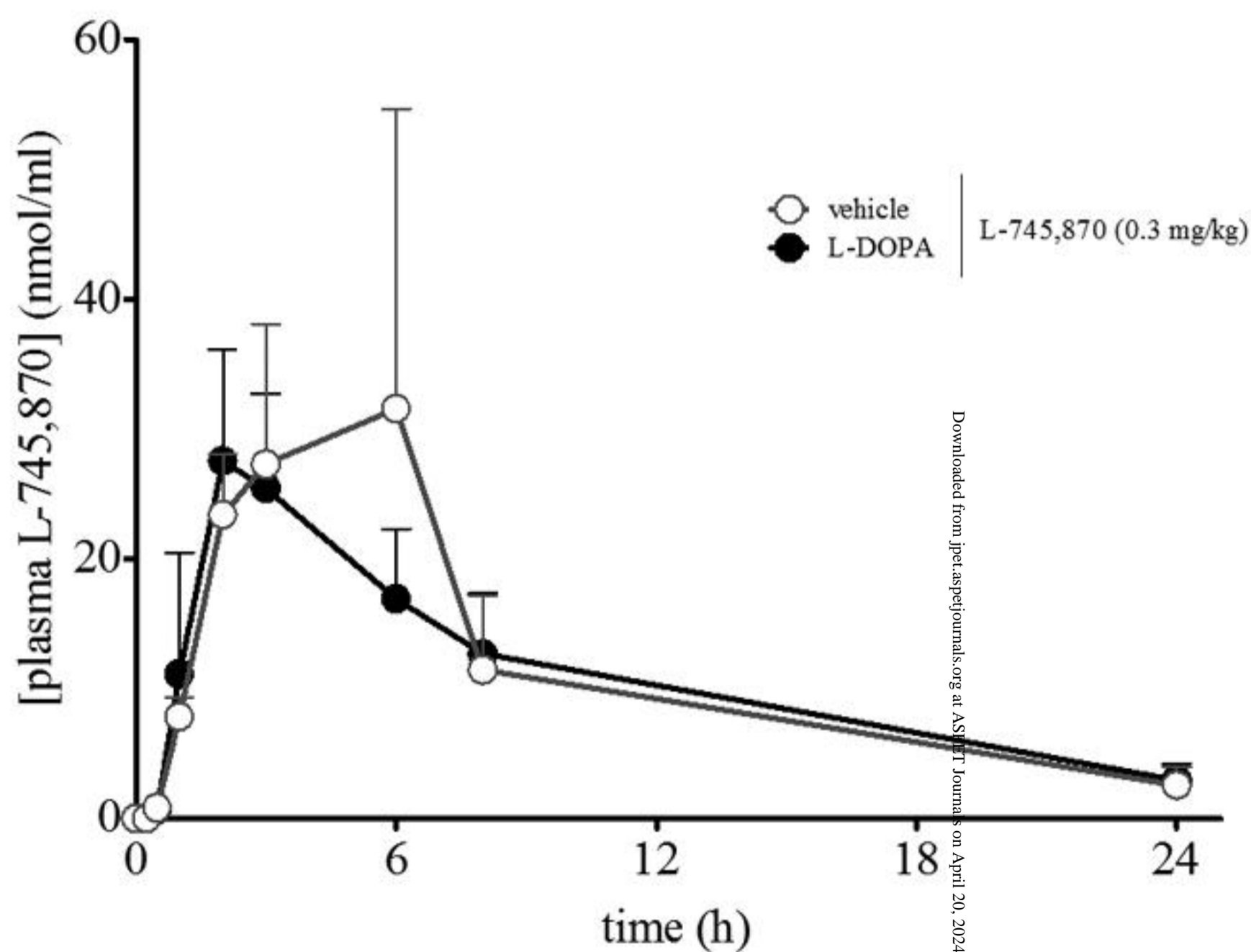


Figure 4



C L-745,870 PK profile - effect of concomitant L-DOPA

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L-745,870	L-DOPA (mg/kg)		significance
	vehicle	30 p.o.	
C_{max} (nM), 0-24h	38.8 ± 19.5	28.6 ± 7.6	$P > 0.05$
AUC_{0-24} (h.nM)	286.5 ± 145.3	266.9 ± 77.6	$P > 0.05$
t_{max} (h)	3.7 ± 1.2	3.3 ± 1.3	$P > 0.05$