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RESEARCH PAPER

Side effect profile of 5-HT treatments for Parkinson's disease and L-DOPA-induced dyskinesia in rats

D Lindenbach, N Palumbo, C Y Ostock, N Vilceus, M M Conti and C Bishop

Behavioral Neuroscience Program, Department of Psychology, Binghamton University – State University of New York, Binghamton, NY, USA

Correspondence
Dr Christopher Bishop, Psychology Department, Binghamton University, PO Box 6000, Binghamton, NY 13901-6000, USA. E-mail: cbishop@binghamton.edu

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BACKGROUND AND PURPOSE

Treatment of Parkinson's disease (PD) with L-DOPA eventually causes abnormal involuntary movements known as dyskinesias in most patients. Dyskinesia can be reduced using compounds that act as direct or indirect agonists of the 5-HT_{1A} receptor, but these drugs have been reported to worsen PD features and are known to produce '5-HT syndrome', symptoms of which include tremor, myoclonus, rigidity and hyper-reflexia.

EXPERIMENTAL APPROACH

Sprague-Dawley rats were given unilateral nigrostriatal dopamine lesions with 6-hydroxydopamine. Each of the following three purportedly anti-dyskinetic 5-HT compounds were administered 15 min before L-DOPA: the full 5-HT_{1A} agonist ±-8-hydroxy-2-dipropylaminotetralin (±8-OH-DPAT), the partial 5-HT_{1A} agonist buspirone or the 5-HT transporter inhibitor citalopram. After these injections, animals were monitored for dyskinesia, 5-HT syndrome, motor activity and PD akinesia.

KEY RESULTS

Each 5-HT drug dose-dependently reduced dyskinesia by relatively equal amounts (±8-OH-DPAT ≥ citalopram ≥ buspirone), but 5-HT syndrome was higher with ±8-OH-DPAT, lower with buspirone and not present with citalopram. Importantly, with or without L-DOPA, all three compounds provided an additional improvement of PD akinesia. All drugs tempered the locomotor response to L-DOPA suggesting dyskinesia reduction, but vertical rearing was reduced with 5-HT drugs, potentially reflecting features of 5-HT syndrome.

CONCLUSIONS AND IMPLICATIONS

The results suggest that compounds that indirectly facilitate 5-HT_{1A} receptor activation, such as citalopram, may be more effective therapeutics than direct 5-HT_{1A} receptor agonists because they exhibit similar anti-dyskinesia efficacy, while possessing a reduced side effect profile.

Abbreviations

5-HIAA, 5-hydroxyindolacetic acid; 6-OHDA, 6-hydroxydopamine; AIMs, abnormal involuntary movements; DOPAC, 3,4-dihydroxyphenylacetic acid; FAS, forepaw adjusting steps; LID, L-DOPA-induced dyskinesia; MAD, median absolute deviation; PD, Parkinson's disease; SSRI, selective 5-HT re-uptake inhibitor

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Tables of Links

TARGETS
5-HT_{1A} receptor
5-HT transporter
D_2 receptor
D_1 receptor

LIGANDS	
8-OH-DPAT	Buspirone
Benserazide	Citalopram
6-Hydroxydopamine	Desipramine
Buprenorphine	L-DOPA

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 (Alexander *et al.*, 2013).

Introduction

Motor disability in Parkinson's disease (PD) is primarily a result of nigrostriatal dopaminergic cell loss, which leads to resting tremor, rigidity, akinesia and postural instability (Jankovic, 2008). At first, PD features are optimally ameliorated by L-DOPA, but long-term use results in the development of debilitating involuntary movements, known as L-DOPA-induced dyskinesia (LID), in ~90% of PD patients (Ahlskog and Muenter, 2001). The cause of LID involves both dys-regulated dopamine release at striatal synapses and hyperphysiological dopamine receptor signal transduction within striatal output neurons (Jenner, 2008; Carta and Bezard, 2011).

Recent evidence suggests that, in the PD brain, a large portion of L-DOPA-derived dopamine is released by 5-HT neurons (Eskow *et al.*, 2009; Huot *et al.*, 2011a; Nevalainen *et al.*, 2011). Lacking D_2 autoreceptors, 5-HT neurons do not have the ability to regulate their own dopamine release, leading to large swings in synaptic dopamine (de la Fuente-Fernandez *et al.*, 2004; Carta *et al.*, 2007). In order to blunt excessive dopamine signalling in the basal ganglia, there has been interest in supplementing L-DOPA therapy with 5-HT_{1A} agonists, which may reduce dyskinesia by activation of 5-HT_{1A} autoreceptors (Kannari *et al.*, 2001; Bezard *et al.*, 2013) and 5-HT_{1A} heteroreceptors (Munoz *et al.*, 2009; Dupre *et al.*, 2011; 2013; Huot *et al.*, 2011b).

While animal models consistently demonstrate that partial and full 5-HT_{1A} agonists reduce LID, the attenuation of LID may coincide with an increase in PD symptoms (Bibbiani *et al.*, 2001; Iravani *et al.*, 2006; Eskow *et al.*, 2007; Gregoire *et al.*, 2009). In humans, the full 5-HT_{1A} agonist sarizotan failed in double-blind clinical trials due to lack of anti-dyskinetic efficacy (Goetz *et al.*, 2007) while the partial 5-HT_{1A} agonist buspirone has been shown to reduce dyskinesia in several smaller-scale trials (n = 5–24; Kleedorfer *et al.*, 1991; Bonifati *et al.*, 1994; Politis *et al.*, 2014). Alternative therapeutic strategies for reducing LID by targeting the 5-HT system have been explored. For example, selective 5-HT re-uptake inhibitors (SSRIs) exhibit anti-LID efficacy at least partially through indirect facilitation of 5-HT_{1A} receptor activation (Bishop *et al.*, 2012; Inden *et al.*, 2012; Conti *et al.*, 2014).

Although often overlooked and not well-studied in animal models, a potentially fatal side effect of many drugs with 5-HT agonist activity is '5-HT syndrome', characterized by tremor, myoclonus, rigidity, punding and hyperreflexia (Jacobs, 1976; Boyer and Shannon, 2005). Many features of 5-HT syndrome, such as tremor and rigidity, are also symptoms of PD, leading to diagnostic confusion (Ener *et al.*, 2003). Thus, in PD patients, 5-HT syndrome from 5-HT_{1A} agonists may often be misdiagnosed as a worsening of PD.

In rats, 5-HT syndrome results from excessive simulation of post-synaptic 5-HT_{1A} receptors in the spinal cord and caudal brain stem (Jacobs and Klemfuss, 1975; Wieland *et al.*, 1989; Lucki, 1992), but in humans, 5-HT syndrome also involves overactivation of 5-HT_{2A} receptors (Boyer and Shannon, 2005; Haberzettl *et al.*, 2013). Direct 5-HT_{1A} agonists robustly produce 5-HT syndrome although high doses of drugs that enhance 5-HT transmission can also produce the syndrome (Smith and Peroutka, 1986; Ener *et al.*, 2003; Haberzettl *et al.*, 2013). Indeed, one of the largest risk factors for manifesting 5-HT syndrome is ingesting multiple drugs that affect the 5-HT system in different ways (Bodner *et al.*, 1995; Boyer and Shannon, 2005). Strikingly, combining L-DOPA with other commonly prescribed PD medications that affect the 5-HT system (such as MAO inhibitors or the D_2 receptor agonist bromocriptine) can produce 5-HT syndrome in humans and animals (Jacobs, 1974; Deakin and Dashwood, 1981; Sandyk, 1986; Heinonen and Myllyla, 1998). Thus, PD patients are at high-risk for manifesting 5-HT syndrome.

We sought to determine if there is a therapeutic dose window for certain 5-HT compounds where LID suppression is achieved without provoking 5-HT syndrome. Additionally, for the first time, we investigated the effects of 5-HT syndrome on motor performance and L-DOPA efficacy, comparing how these effects differ in a parkinsonian versus healthy motor system. The results show that all three 5-HT compounds tested exhibited similar anti-LID efficacy, but motor side effects, including 5-HT syndrome, were more prevalent with 5-HT_{1A} agonists than with a SSRI, suggesting that a SSRI may be a superior anti-LID agent.

Methods

Animals

Adult male Sprague-Dawley rats were used (n = 22; Harlan, Indianapolis, IN, USA). Rats were pair-housed in plastic cages

and given free access to water and standard laboratory rat food. The colony room was maintained at 22–23°C on a 12 h light/dark cycle (lights on 07:00 h). The experiments were approved by the Institutional Animal Care and Use Committee of Binghamton University and throughout the study, the animals were cared for in full accordance with the guidelines of this committee and that of the National Institutes of Health 'Guide for the Care and Use of Laboratory Animals'. All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010).

Surgery

Rats were given a unilateral dopamine lesion to the left medial forebrain bundle. Prior to surgery, rats were given injections of desipramine (25 mg·kg^{-1}) to protect noradrenaline (NA) neurons and buprenorphine (0.03 mg·kg^{-1}) as a pre-emptive analgesia. Animals were then anaesthetized with ~2% isoflurane (Baxter Healthcare, Deerfield, IL, USA) mixed with oxygen (1 L·min^{-1}); depth of anaesthesia was assessed by loss of consciousness and a lack of response to simple nociceptive stimuli. A 26-gauge needle was lowered into the target site: posterior 1.8 mm, lateral 2.0 mm and ventral –8.6 mm from bregma (Paxinos and Watson, 1998). Subsequently, 6-OHDA (12 µg in 4 µL) was injected at a constant flow rate for 2 min and timed to begin 30 min after desipramine injection. The needle was withdrawn 5 min later.

5-HT syndrome scale

We codified the signs of rat 5-HT syndrome using criteria from a review by Haberzettl *et al.* (2013). Rats were observed in clear, plastic cylinders and rated by a trained observer (≥95% agreement between experimenters). Rats were rated for the presence of (i) lower lip retraction; (ii) flat body posture; (iii) forepaw treading; (iv) resting tremor; (v) straub tail; (vi) hindlimb abduction; and (vii) lateral head-weaving. The scale rated each subtype as 0 (not present) or 1 (present).

We attempted to measure the severity of each subtype (as in Smith and Peroutka, 1986 or Wieland *et al.*, 1989), but did not observe noticeable severity differences for all subtypes. Given this, subtypes were scored dichotomously, so that each subtype exerted proportional effects on the total severity score.

Abnormal involuntary movements (AIMs) scale

The AIMs test is a measure of dyskinesia. Rats were monitored for AIMs using a procedure modified from Cenci and Lundblad (2007) and described in detail in Lindenbach *et al.* (2011). Rats were observed in clear plastic cylinders and were rated by a trained observer (≥95% agreement between experimenters). During each rating period, individual dyskinesia severity scores ranging from 0 (not present) to 4 (severe and not interruptible) were given for axial, limb and orolingual dyskinesias. The three AIM subtypes were summed to create a single AIMs score for data analysis.

Forepaw adjusting steps (FAS) test

The FAS test is a measure of akinesia, a cardinal symptom of PD and rats with >80% dopamine depletion perform poorly on the test (Chang *et al.*, 1999). L-DOPA reduces this deficit so the test can be used to determine if an adjunct is interfering with L-DOPA efficacy (Eskow *et al.*, 2007). To perform the test, an experimenter held the rat's hindlimbs and one forelimb such that the free forelimb was forced to bear the rat's body weight. For each trial, rats were moved laterally for 90 cm over 10 s, and the number of laterally adjusting steps was counted by a trained observer (≥95% agreement among experimenters). Each session consisted of three trials in each direction for a total of six trials with each limb. Higher stepping numbers are considered to indicate greater motor performance.

Motion chamber measurements

Motion chambers allow assessment of drug-induced changes in spontaneous motor activity. Locomotor activity was assessed by infrared photocell arrays in six identical acrylic chambers measuring 41 × 41 × 30.5 cm (Accuscan Instruments, Columbus, OH, USA). The software analysed patterns of photo beam breaks to measure horizontal and vertical movements (Hillegaart *et al.*, 1989; Lindenbach *et al.*, 2011).

General procedure

Figure 1 contains a flow diagram of the procedure used in the present study. Three weeks after surgery, all rats were injected with L-DOPA (12 mg·kg^{-1}) daily for 7 days in order to 'prime' them for maximal behavioural response to future L-DOPA injections (Cenci and Lundblad, 2007; Bhide *et al.*, 2013). Two days later, they were given the test dose of L-DOPA (6 mg·kg^{-1}) and monitored for dyskinesia using the AIMs scale; only rats displaying ≥25 AIMs were included in the study (22 of 24 rats). Rats were run in two cohorts with the order of experiments counterbalanced between cohorts, although experiment 2A was only run with the second cohort (after completing all other experiments). In all cases, behavioural testers were blind to treatment. In between performing experiments 1, 2A, 2B and 3, rats were given two injections of L-DOPA (6 mg·kg^{-1}) to maintain maximal L-DOPA sensitivity. Rats participated in experiments 2–3 days per week on non-consecutive days.

Experiment 1: effects of 5-HT$_{1A}$ receptor activation on 5-HT syndrome and LID

Using a counterbalanced, within-subjects design, rats were injected with the full 5-HT$_{1A}$ agonist ±-8-hydroxy-2-dipropylaminotetralin (±8-OH-DPAT; 0.3 and 1 mg·kg^{-1}), the partial 5-HT$_{1A}$ agonist buspirone (1 and 3 mg·kg^{-1}), the SSRI citalopram (2 and 5 mg·kg^{-1}) or their common vehicle. Rats were monitored for 5-HT syndrome for 1 min every 10 min for the next 180 min. Thus, the maximal 5-HT syndrome severity score was seven for each time point (collapsed across subtype), 18 for each subtype (collapsed across time) and 126 for each session (collapsed across subtype and time). At 15 min post-injection, they were administered L-DOPA (6 mg·kg^{-1}) and monitored for dyskinesia using the AIMs scale for 1 min every 10 min for the next 180 min. Thus, rats were monitored for 5-HT syndrome and AIMs during the same session, with alternating measurements from each scale taken 5 min apart from each other.

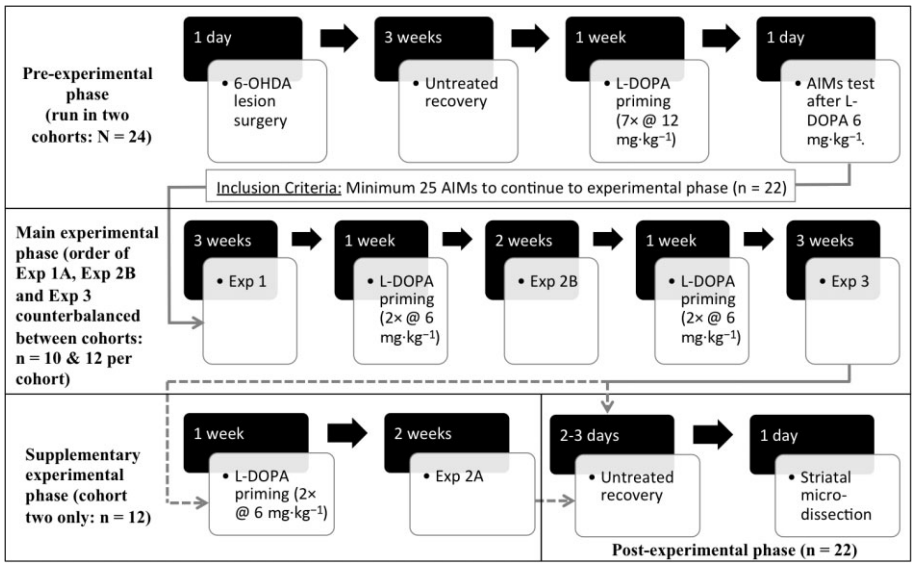

Figure 1

Flow diagram illustrating the experimental procedure. The experiment lasted 4–5 months and was run in two cohorts of 12 rats each, with the cohorts beginning the experiment ~1 month apart. After the 6-OHDA lesion and L-DOPA priming, 22 rats expressed sufficient AIMs to be included in the main experimental phase. In order to minimize tolerance to 5-HT treatments while maintaining sensitivity to L-DOPA, experiments were separated from each other by 1 week, during which time rats only received L-DOPA. After completion of all experiments, rats were rapidly decapitated and striata were microdissected for monoamine analysis.

Experiment 2A: effects of 5-HT$_{1A}$ receptor activation on motor performance OFF L-DOPA

Using a counterbalanced, within-subjected design, rats were injected with ±8-OH-DPAT (1 mg·kg^{-1}), buspirone (3 mg·kg^{-1}), citalopram (5 mg·kg^{-1}) or vehicle. Fifteen minutes later, they were injected with L-DOPA's vehicle. Motor performance was assessed using the FAS test at 45 and 135 min after 5-HT drug injection.

Experiment 2B: effects of 5-HT$_{1A}$ activation on motor performance ON L-DOPA

Using a counterbalanced, within-subjected design, rats were injected with ±8-OH-DPAT (1 mg·kg^{-1}), buspirone (3 mg·kg^{-1}), citalopram (5 mg·kg^{-1}) or vehicle. Fifteen minutes later, they were injected with L-DOPA (6 mg·kg^{-1}). Motor performance was assessed using the FAS test at 45 and 135 min after 5-HT drug injection.

Experiment 3: effects of 5-HT$_{1A}$ activation on motor activity ON and OFF L-DOPA

Rats were habituated to the motion chambers for 180 min on 4 days within 1 week. Using a counterbalanced, within-subjected design, rats were injected with ±8-OH-DPAT (1 mg·kg^{-1}), buspirone (3 mg·kg^{-1}), citalopram (5 mg·kg^{-1}) or vehicle. Fifteen minutes later, they were injected with L-DOPA (0 and 6 mg·kg^{-1}). Rats were placed in the motion chambers immediately after the second injection and spontaneous horizontal and vertical activity was monitored continuously for 180 min.

Monoamine tissue analysis

Following completion of all experiments, rats were left untreated for at least 2 days. Subsequently, rats were rapidly decapitated, both striata were microdissected and the tissue was analysed via HPLC with electrochemical detection. We analysed striatal tissue concentrations of dopamine, the dopamine metabolite 3,4-dihydroxyphenylacetic acid (DOPAC), NA, 5-HT and the 5-HT metabolite 5-hydroxyindolacetic acid (5-HIAA). The protocol was based on Kilpatrick *et al.* (1986) and described in detail in Lindenbach *et al.* (2011). Samples were homogenized in perchloric acid and standards were run across a range of 10^{-6} to 10^{-9} M. Values were adjusted to wet tissue weights.

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS v20 (IBM, Chicago, IL, USA) with α set at 0.05. As the AIMs and 5-HT syndrome scales use ordinal intervals, we present the data as medians with median absolute deviation (MAD) as our variance estimate. These data were analysed with the non-parametric Friedman test and Wilcoxon sign–rank contrasts. All other data were analysed using ANOVAs and *t*-test contrasts. When using ANOVAs, if Mauchley's test of sphericity showed significant heteroscedasticity ($P < 0.05$), we used Huyhn–Feldt degrees of freedom corrections. For all planned contrasts (parametric and non-parametric), α was adjusted for multiple comparisons using the Dunn–Sidak method.

Drugs

Desipramine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in dH_2O. Buprenorphine hydrochloride (Hospira, Lake Forest, IL, USA) was dissolved in saline (0.9% NaCl). L-DOPA methyl ester hydrochloride (Sigma-Aldrich) and 6-hydroxydopamine hydrobromide (6-OHDA; Sigma-Aldrich) were dissolved in saline with 0.1% ascorbic acid. The

peripheral decarboxylase inhibitor benserazide hydrochloride (Sigma-Aldrich) was co-administered in the same vehicle as L-DOPA at a dosage of 15 mg·kg^{-1}. Buspirone hydrochloride (Sigma-Aldrich), ±8-OH-DPAT hydrobromide (Sigma-Aldrich) and citalopram hydrobromide (LKT Laboratories, St. Paul, MN, USA) were dissolved in 20% DMSO and 80% dH_2O. Systemic administrations were performed s.c. except for desipramine and buprenorphine, which were given i.p.

Results

Monoamine tissue analysis

Table 1 shows the mean striatal monoamine content for all rats in this study. Raw monoamine values were considered to be outliers and removed from subsequent analyses if they differed from the group mean by more than three SDs. Comparing the lesioned striata to the non-lesioned side, tissue dopamine content was reduced by 98% ($t_{19} = 16.77$, $P < 0.001$) and DOPAC content was reduced by 97% ($t_{20} = 7.74$, $P < 0.001$). Despite administration of desipramine prior to 6-OHDA, there was a 62% depletion of striatal NA relative to the intact hemisphere ($t_{18} = 2.42$, $P = 0.026$). Also on the lesioned side, 5-HT was reduced by 12% ($t_{20} = 3.51$, $P = 0.002$) while 5-HIAA was increased by 21% ($t_{21} = 2.31$, $P = 0.031$).

Experiment 1: effects of 5-HT$_{1A}$ activation on 5-HT syndrome and LID

Table 2 shows the median frequency of each sign of 5-HT syndrome after each drug. The full 5-HT$_{1A}$ agonist ±8-OH-DPAT elicited all seven subtypes during testing although lateral head-weaving and hindlimb abduction were rare. Buspirone caused lower lip retraction, flat body posture, forepaw treading, resting tremor, straub tail and hindlimb abduction, but lateral head-weaving was never observed. When given citalopram, 5-HT syndrome behaviours were rare, but included flat body posture, straub tail and resting tremor; these behaviours occurred in 1 of 20 rats given 2 mg·kg^{-1} and in 2 of 20 rats given 5 mg·kg^{-1} with a maximal individual 5-HT syndrome score of 3. No 5-HT syndrome was observed in any animals after an injection of vehicle + L-DOPA.

An omnibus Friedman test indicated significant 5-HT syndrome induction with certain treatments ($\chi^2 = 116.40$, $P < 0.001$). Using the Wilcoxon sign–rank test, ±8-OH-DPAT and buspirone dose-dependently increased 5-HT syndrome (all $Z \geq 3.25$, $P \leq 0.001$) while citalopram did not induce 5-HT syndrome at either dose. At the low dose we tested, 5-HT syndrome was greater after ±8-OH-DPAT 0.3 mg·kg^{-1} than after buspirone 1 mg·kg^{-1} ($Z = 3.93$, $P < 0.001$). Likewise, 5-HT syndrome was more severe with ±8-OH-DPAT 1 mg·kg^{-1} than with buspirone 3 mg·kg^{-1} ($Z = 3.89$, $P < 0.001$).

All three 5-HT drugs dose-dependently reduced AIMs scores compared with L-DOPA alone (Table 2; all $P < 0.001$). Comparing among drugs at the low dose, AIMs reduction was equivalent except that ±8-OH-DPAT 0.3 mg·kg^{-1} reduced AIMs more than buspirone 1 mg·kg^{-1} ($Z = 3.03$, $P = 0.002$). Examining the high dose, ± 8-OH-DPAT 1 mg·kg^{-1} showed greater AIMs suppression than both buspirone 3 mg·kg^{-1} ($Z = 2.99$, $P = 0.003$) and citalopram 5 mg·kg^{-1} ($Z = 3.13$, $P = 0.002$).

In order to assess the degree to which 5-HT syndrome and LID suppression temporally coincide, we examined the time course of each behaviour. For rats given the 0.3 mg·kg^{-1} dose of ±8-OH-DPAT, 5-HT syndrome lasted for 140 min (Figure 2A) and dyskinesia suppression lasted until 145 min (Figure 2D; all $P < 0.001$). With 1 mg·kg^{-1} ±8-OH-DPAT, 5-HT syndrome lasted the entire 180 min rating period (Figure 2A), while dyskinesia suppression lasted only until 155 min (Figure 2D; all $P < 0.001$). Buspirone 1 mg·kg^{-1} caused 5-HT syndrome for 70 min (Figure 2B), while reducing AIMs until 85 min (Figure 2E; all $P < 0.001$). The 3 mg·kg^{-1} dose of buspirone caused 5-HT syndrome for 130 min (Figure 2B) while suppressing AIMs until 145 min (Figure 2E; all $P < 0.001$). As noted, citalopram did not induce significant 5-HT syndrome compared with vehicle (Figure 2C), but 2 mg·kg^{-1} reduced AIMs until 115 min while 5 mg·kg^{-1} reduced AIMs until 155 min (Figure 2F; all $P < 0.001$).

Experiment 2A: effects of 5-HT$_{1A}$ activation on motor performance OFF L-DOPA

The FAS test was used to determine how 5-HT drugs (and associated 5-HT syndrome) affect baseline motor performance in the intact and dopamine-lesioned forelimb. Data were analysed using a three-way repeated-measures ANOVA: 2 (Lesion) × 4 (Treatment) × 2 (Time). A main effect of lesion indicated decreased stepping with the lesioned-side forelimb ($F_{1,11} = 1063.69$, $P < 0.001$, $\eta_p^2 = 0.990$). 5-HT treatment also impacted stepping ($F_{3,33} = 83.75$, $P < 0.001$, $\eta_p^2 = 0.884$). Importantly for our planned comparisons, the Lesion × Treatment × Time interaction was significant ($F_{3,33} = 10.09$, $P < 0.001$, $\eta_p^2 = 0.479$).

Therefore, we first examined stepping with the intact forelimb to determine if the 5-HT drugs impacted non-parkinsonian motor performance (Figure 3A). ±8-OH-DPAT 1 mg·kg^{-1} increased intact-side stepping at both 45 min

Table 1

Mean striatal monoamine content of dopamine, DOPAC, NA, 5-HT and 5-HIAA

	Dopamine (pg·mg^{-1})	DOPAC (pg·mg^{-1})	NA (pg·mg^{-1})	5-HT (pg·mg^{-1})	5-HIAA (pg·mg^{-1})
Intact striatum	6748 ± 399	3206 ± 401	181 ± 48	511 ± 26	816 ± 64
Lesioned striatum	117* ± 16	84* ± 7	69* ± 21	404* ± 22	916* ± 71

Results are presented as mean ± SEM. Striatal tissue ($n = 22$) was homogenized and monoamine concentrations were determined by HPLC with values expressed as pg of monoamine mg^{-1} of tissue. *$P < 0.05$ versus intact striatum.

Table 2
Median sum of 5-HT syndrome and AIMs scores (± MAD) after injection of 5-HT compounds and L-DOPA

	5-HT syndrome								LID	
	Total 5-HT syndrome score	Lower lip retraction	Flat body posture	Forepaw treading	Straub tail	Resting tremor	Hindlimb abduction	Lateral head-weaving	Total AIMs score	
Vehicle	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	64 ± 7.5	
±8-OH-DPAT 0.3 mg·kg^{-1}	35* ± 2.5	15* ± 1	11* ± 1	5.5* ± 2	2* ± 1	1 ± 1	0 ± 0	0 ± 0	24* ± 5	
±8-OH-DPAT 1 mg·kg^{-1}	57.5* ± 3.5	18* ± 0	16* ± 1	11* ± 2	3* ± 2	7* ± 1.5	0.5 ± 0.5	0 ± 0	5* ± 2	
Buspirone 1 mg·kg^{-1}	12.5* ± 3.5	7* ± 2	6* ± 2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	29.5* ± 7.5	
Buspirone 3 mg·kg^{-1}	23* ± 2.5	13* ± 2	9* ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	12* ± 6	
Citalopram 2 mg·kg^{-1}	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	30* ± 9	
Citalopram 5 mg·kg^{-1}	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	11.5* ± 6.5	

Rats (n = 20) were injected with ±8-OH-DPAT, buspirone or citalopram and rated for 5-HT syndrome for 1 min every 10 min over 180 min. Fifteen minutes after 5-HT drug injection, all rats received L-DOPA (6 mg·kg^{-1}) and were rated for AIMs for 1 min every 10 min over 180 min.
*P < 0.001 versus vehicle.

(t_{11} = 6.34, P < 0.001) and 135 min post-injection (t_{11} = 6.78, P < 0.001). Buspirone 3 mg·kg^{-1} did not change intact-limb performance at 45 min (t_{11} = 1.30, P = 0.220), but it decreased the number of steps taken at 135 min (t_{11} = 5.81, P < 0.001). Citalopram 5 mg·kg^{-1} did not significantly affect intact-side stepping at either time point. With the lesioned forelimb (Figure 3B), ±8-OH-DPAT 1 mg·kg^{-1} increased stepping at 45 min (t_{11} = 12.63, P < 0.001) and 135 min (t_{11} = 7.74, P < 0.001). At 45 min post-injection, lesioned-side stepping was improved by buspirone 3 mg·kg^{-1} (t_{11} = 7.58, P < 0.001) and citalopram 5 mg·kg^{-1} (t_{11} = 4.43, P = 0.001), but the effects were not statistically significant at 135 min.

Experiment 2B: effects of 5-HT$_{1A}$ activation on motor performance ON L-DOPA

We next examined how motor performance was impacted by an interaction between the 5-HT drugs and L-DOPA, using a three-way repeated-measures ANOVA: 2 (Lesion) × 5 (Treatment) × 2 (Time). Lesion reduced the number of steps taken ($F_{1,21}$ = 194.44, P < 0.001 η_p^2 = 0.903) while treatment dynamically altered stepping ($F_{3.0,61.9}$ = 73.95, P < 0.001 η_p^2 = 0.779). There was a significant interaction of Lesion × Treatment × Time ($F_{4,84}$ = 11.45, P < 0.001, η_p^2 = 0.353).

When using the intact forelimb (Figure 4A), L-DOPA did not significantly affect stepping at either time point. L-DOPA combined with ±8-OH-DPAT 1 mg·kg^{-1} increased stepping relative to L-DOPA alone at 45 min (t_{21} = 5.07, P < 0.001) and 135 min post-injection (t_{21} = 8.94, P < 0.001). By contrast, buspirone 3 mg·kg^{-1} combined with L-DOPA decreased stepping relative to L-DOPA alone at 45 min (t_{21} = 4.99, P < 0.001) and 135 min (t_{21} = 4.52, P < 0.001). Compared with L-DOPA alone, citalopram 5 mg·kg^{-1} did not significantly alter intact-side stepping.

Analysing the lesioned forelimb (Figure 4B), L-DOPA did not significantly increase stepping at the 45 min time point (30 min post L-DOPA; t_{21} = 1.02, P = 0.319), but there was a significant improvement at the 135 min time point (120 min post L-DOPA; t_{21} = 3.62, P = 0.002). Compared with L-DOPA alone, adding ±8-OH-DPAT 1 mg·kg^{-1} augmented stepping at 45 min (t_{21} = 13.71, P < 0.001) and 135 min (t_{21} = 5.83, P < 0.001). The combination of buspirone 3 mg·kg^{-1} and L-DOPA increased stepping relative to L-DOPA monotherapy at 45 min (t_{21} = 6.94, P < 0.001). Relative to L-DOPA alone, adding citalopram 5 mg·kg^{-1} improved stepping at 45 min (t_{21} = 2.98, P = 0.007), but this was not considered statistically significant given our Dunn-Sidak α adjustment.

Experiment 3: effects of 5-HT$_{1A}$ activation on motor activity ON and OFF L-DOPA

We finally examined the effects of 5-HT compounds and L-DOPA on horizontal and vertical movements in an open chamber using a two-way repeated-measures ANOVA: 2 (L-DOPA treatment) × 4 (5-HT treatment). L-DOPA treatment increased horizontal activity relative to vehicle ($F_{1,21}$ = 55.33, P < 0.001, η_p^2 = 0.725). 5-HT treatment also impacted horizontal activity ($F_{2.3,47.7}$ = 27.36, P < 0.001, η_p^2 = 0.566) and there was an interaction between the two factors ($F_{1.8,38.7}$ = 23.29, P < 0.001, η_p^2 = 0.526). Compared with vehicle, horizontal activity increased after monotherapy with either L-DOPA (t_{21} = 6.75, P < 0.001) or ±8-OH-DPAT 1 mg·kg^{-1}

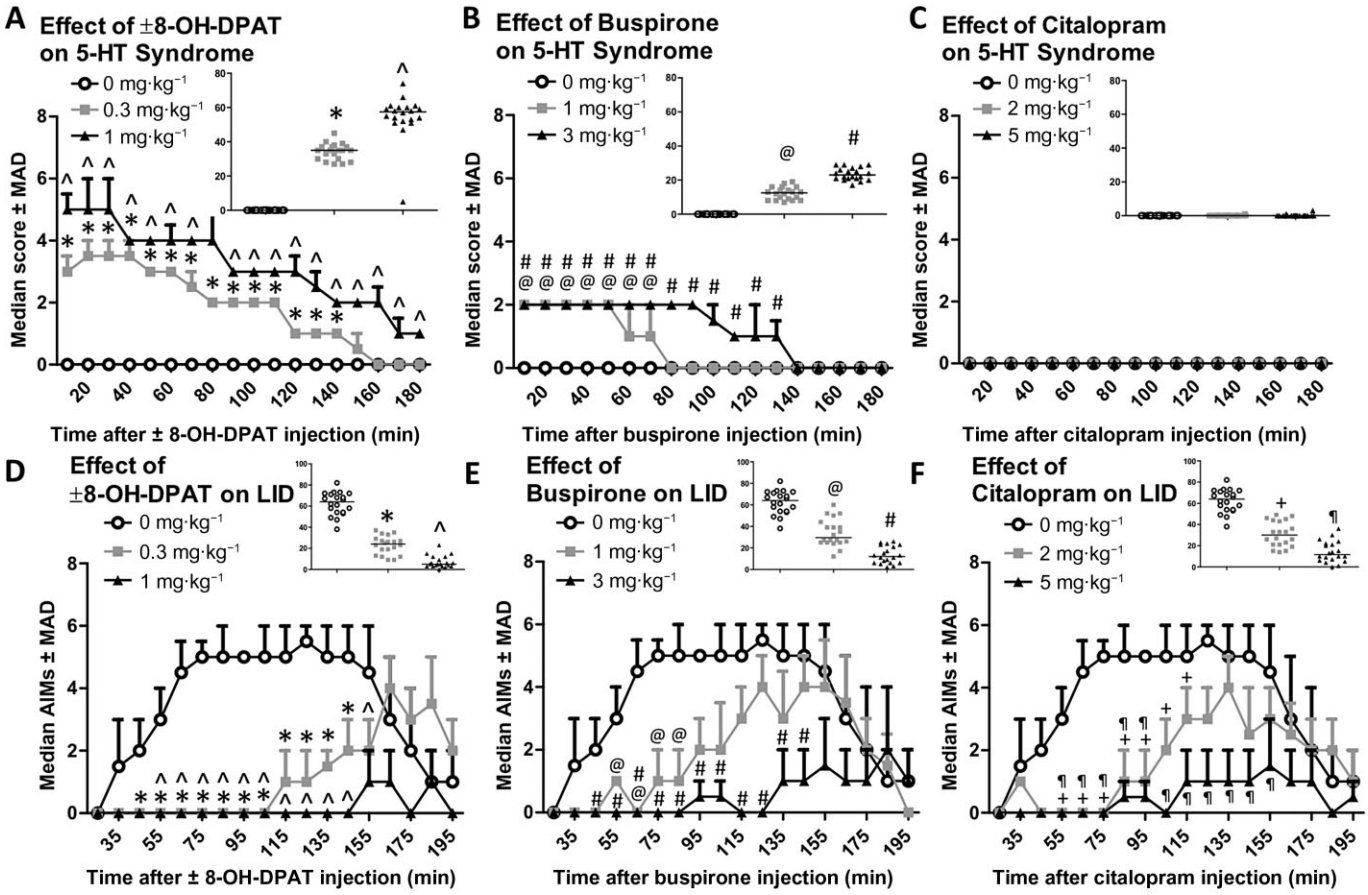

Figure 2

Temporal relationship between the induction of 5-HT syndrome and the suppression of LID. Before the injection of L-DOPA, rats (n = 20) were pretreated with (A) ±8-OH-DPAT (0.3 and 1 mg·kg^{-1}), (B) buspirone (1 and 3 mg·kg^{-1}), (C) citalopram (2 and 5 mg·kg^{-1}) or their vehicle and rated for 5-HT syndrome for 1 min every 10 min for the next 180 min. For each panel, symbols depict the median 5-HT syndrome severity score (± MAD) at each time point. Inset graphs show the total 5-HT syndrome score for each rat with each treatment with a horizontal line marking the median score. (D–F) Fifteen minutes after pretreatment, all rats were injected with L-DOPA (6 mg·kg^{-1}) and rated for LID for 1 min every 10 min for the next 180 min. Inset graphs denote total AIMs score for each rat with each treatment with a horizontal line marking the median score. *P < 0.001 ± 8-OH-DPAT 0.3 mg·kg^{-1} versus 0 mg·kg^{-1}; ^P < 0.001 ± 8-OH-DPAT 1 mg·kg^{-1} versus 0 mg·kg^{-1}; @P < 0.001 buspirone 1 mg·kg^{-1} vs. 0 mg·kg^{-1}; #P < 0.001 buspirone 3 mg·kg^{-1} versus 0 mg·kg^{-1}; +P < 0.001 citalopram 2 mg·kg^{-1} versus 0 mg·kg^{-1}; ¶P < 0.001 citalopram 5 mg·kg^{-1} versus 0 mg·kg^{-1}.

(t_{21} = 3.15, P = 0.005). Relative to L-DOPA alone, decreased horizontal activity was seen with the addition of ±8-OH-DPAT 1 mg·kg^{-1} (t_{21} = 4.99, P < 0.001), buspirone 3 mg·kg^{-1} (t_{21} = 5.86, P < 0.001) or citalopram 5 mg·kg^{-1} (t_{21} = 6.76, P < 0.001). Compared with each 5-HT drug alone, the addition of L-DOPA increased horizontal activity for buspirone 3 mg·kg^{-1} (t_{21} = 6.63, P < 0.001), but the increase was not significant for ±8-OH-DPAT 1 mg·kg^{-1} (t_{21} = 0.74, P = 0.467) or citalopram 5 mg·kg^{-1} (t_{21} = 2.55, P = 0.019) given the multiple comparisons corrections.

We also examined vertical activity using the same two-way repeated-measures ANOVA. We observed main effects of L-DOPA treatment ($F_{1,21}$ = 23.20, P < 0.001, η_p^2 = 0.525) and 5-HT drug ($F_{1.6,33.2}$ = 49.70, P < 0.001, η_p^2 = 0.703) as well as an interaction ($F_{1.6,33.6}$ = 10.79, P = 0.001, η_p^2 = 0.339). L-DOPA alone increased vertical activity relative to vehicle (t_{21} = 4.08, P = 0.001). Compared with vehicle, reduced vertical activity was seen with ±8-OH-DPAT 1 mg·kg^{-1} (t_{21} = 6.32, P < 0.001), buspirone 3 mg·kg^{-1} (t_{21} = 5.99, P < 0.001) and citalopram 5 mg·kg^{-1} (t_{21} = 3.96, P < 0.001). Likewise, compared with L-DOPA alone, there was a reduction in vertical activity after the addition of ±8-OH-DPAT 1 mg·kg^{-1} (t_{21} = 6.77, P < 0.001), buspirone 3 mg·kg^{-1} (t_{21} = 5.57, P < 0.001) or citalopram 5 mg·kg^{-1} (t_{21} = 4.57, P < 0.001). Relative to monotherapy with a 5-HT drug, the addition of L-DOPA increased vertical activity with buspirone (t_{21} = 4.64, P < 0.001), but this increase was not significant with ±8-OH-DPAT (t_{21} = 0.48, P = 0.637) or citalopram (t_{21} = 2.49, P = 0.021).

Discussion and conclusions

The present research compared the efficacy and side-effect profiles of 5-HT compounds known to reduce LID at least partially through 5-HT$_{1A}$ receptor activation. At the doses we tested, the 5-HT treatments provided relatively similar anti-

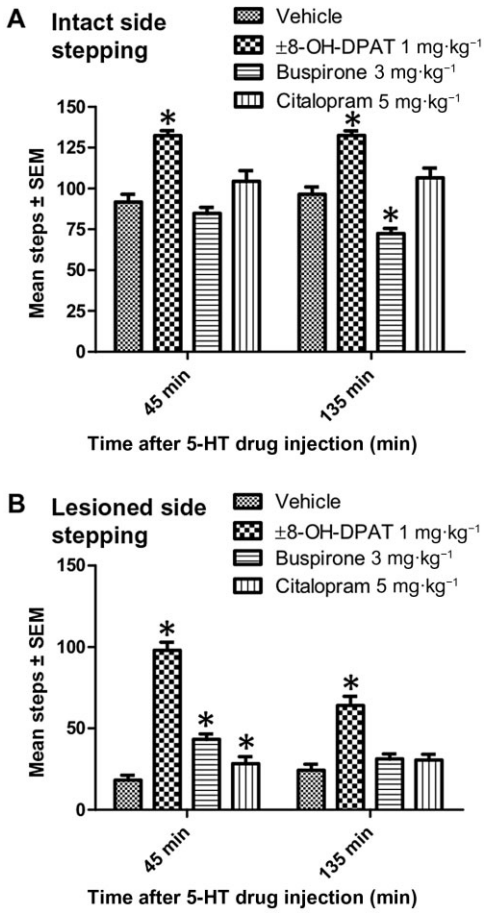

Figure 3

Motor performance on the FAS test after monotherapy with ±8-OH-DPAT (1 mg·kg^{-1}), buspirone (3 mg·kg^{-1}), citalopram (5 mg·kg^{-1}) or their vehicle. Fifteen minutes after this injection, they were treated with vehicle. Rats (n = 12) were tested at 45 and 135 min after 5-HT drug injection. (A) Total number of steps with the 'intact side' forelimb. (B) Total number of steps with the 'lesioned side' forelimb. *P < 0.01 versus vehicle.

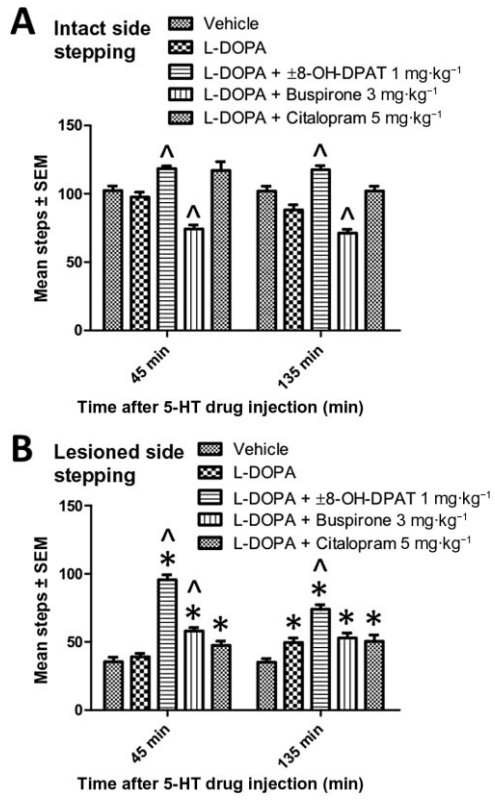

Figure 4

Motor performance on the FAS test after co-treatment with 5-HT compounds and L-DOPA. Rats (n = 22) were injected with ±8-OH-DPAT (1 mg·kg^{-1}), buspirone (3 mg·kg^{-1}), citalopram (5 mg·kg^{-1}) or their vehicle. Fifteen minutes later, they were given L-DOPA (6 mg·kg^{-1}) or vehicle. The FAS test was performed at 45 and 135 min after the first injection. (A) Total number of steps with the 'intact side' forelimb. (B) Total number of steps with the 'lesioned side' forelimb. *P < 0.01 versus vehicle; ∧P < 0.01 versus L-DOPA.

LID efficacy (although ±8-OH-DPAT ≥ citalopram ≥ buspirone), but 5-HT syndrome was most severe with the full 5-HT$_{1A}$ agonist ±8-OH-DPAT, milder with the partial 5-HT$_{1A}$ agonist buspirone and generally absent with the SSRI citalopram. Each 5-HT drug provided an improvement of akinesia on the FAS test, but also reduced spontaneous rearing behaviours in the motion chambers.

Relationship between 5-HT syndrome and LID

This is the first study to systematically examine 5-HT syndrome in an animal model of PD or LID. A key goal of the present study was to examine the temporal relationship between the expression of 5-HT syndrome and LID. In line with previous research, the full 5-HT$_{1A}$ agonist ±8-OH-DPAT potently attenuated LID (Bibbiani *et al.*, 2001; Iravani *et al.*, 2006), but 5-HT syndrome lasted as long or longer than LID suppression at each dose tested (cf. Figure 2A and D). The partial 5-HT$_{1A}$ agonist buspirone also significantly reduced LID and induced 5-HT syndrome although the magnitude was reduced (cf. Figure 2B and E). The present results thus suggest that direct 5-HT$_{1A}$ agonist compounds are less favourable as viable L-DOPA adjuncts as they reduce dyskinesia at the cost of provoking 5-HT syndrome. Interestingly, human studies showing LID reductions with buspirone do not mention 5-HT syndrome by name (Kleedorfer *et al.*, 1991; Bonifati *et al.*, 1994) although some of the clinical descriptions of buspirone's side effects are commensurate with 5-HT syndrome (Politis *et al.*, 2014). Given this discrepancy between the human and rat literature, it is possible that buspirone has a lower 5-HT syndrome liability in humans than it does in rats.

In contrast to the 5-HT$_{1A}$ agonists tested, the SSRI citalopram reduced LID by amounts comparable with ±8-OH-DPAT and buspirone without provoking 5-HT syndrome. While citalopram clearly exhibits some anti-LID efficacy through activation of 5-HT$_{1A}$ receptors as its efficacy can be partially blocked by the 5-HT$_{1A}$ antagonist WAY100635 (Inden *et al.*, 2012; Conti *et al.*, 2014), citalopram may modify L-DOPA's actions through additional mechanisms. For example, by

enhancing endogenous 5-HT, citalopram may also stimulate 5-HT_{1B} receptors known to reduce L-DOPA- and D_1 receptor-stimulated dyskinesia (Munoz *et al.*, 2008; 2009; Jaunarajs *et al.*, 2009; Bezard *et al.*, 2013). A second possibility is that citalopram has a lower 5-HT syndrome liability since SSRIs increase 5-HT tone overall while maintaining endogenous control of 5-HT_{1A} activation.

The interaction of L-DOPA with 5-HT drugs should be explored in future studies. As L-DOPA potentiates 5-HT syndrome induced by an MAO-I (Deakin and Dashwood, 1981; Heinonen and Myllyla, 1998), L-DOPA may also increase 5-HT syndrome caused by an SSRI or a 5-HT_{1A} agonist. Additionally, co-expression of 5-HT syndrome and LID may interfere with an experimenter's ability to code each set of behaviours independently. While this possibility is difficult to test empirically, in the present study, the pattern of severity for 5-HT syndrome (±8-OH-DPAT > buspirone > citalopram) did not follow the same pattern as for LID suppression (±8-OH-DPAT ≥ citalopram ≥ buspirone) indicating that the two measurements exhibit some degree of independence.

Effect of 5-HT syndrome on motor performance and spontaneous motor activity

Experimental evidence from animal models has suggested that stimulation of 5-HT receptors may provide anti-parkinsonian benefit (Bishop *et al.*, 2004; Mignon and Wolf, 2007; Dupre *et al.*, 2008; Li *et al.*, 2013); however, these studies have rarely considered concurrent side effects. In this study, when injected alone, high doses of ±8-OH-DPAT, buspirone and citalopram all increased stepping with the lesioned forelimb during the FAS test (Figure 3). This is congruous with previous research suggesting that 5-HT_{1A} activation counters akinesia or catalepsy brought about by dopamine deprivation (Prinssen *et al.*, 1999; Loane and Politis, 2012). Although 5-HT syndrome was present during the FAS test (cf. Figures 2A and 3A) it is not clear whether such increases reflected a side effect of 5-HT_{1A} receptor stimulation or general motor activating properties (Figure 5A). In fact, stimulation of striatal 5-HT_{1A} receptors may modify glutamate signalling, providing anti-PD benefit orthogonal to the effects of L-DOPA.

While most studies find that there is a therapeutic window for 5-HT_{1A} agonists to relieve LID without worsening PD features, no therapeutic range was found by Iravani *et al.* (2006), who reported that +8-OH-DPAT increased PD motor disability on its own or when combined with L-DOPA. While Iravani *et al.* used the more potent + enantiomer (rather than the racemic mixture used in the present study), our findings are similar in that we report that ±8-OH-DPAT caused postural disturbances (i.e. flat body posture: Table 2) altered patterns of spontaneous movement and suppressed some of the pro-motor effects of L-DOPA (Figure 5). Given the behavioural similarities between PD and 5-HT syndrome (Ener *et al.*, 2003; Jankovic, 2008), it is possible that with many rating scales, 5-HT syndrome symptoms could be codified as PD symptoms.

Despite increased lesioned-side stepping, buspirone decreased intact-side stepping at the 135 min time point (Figures 1B and 2). This apparent motor suppression may be due to the fact that buspirone is a full antagonist at D_2, D_3 and D_4 receptors (k_i in the nanomolar range) and that several of buspirone's metabolites also possess high affinity for the D_3 and D_4 receptors (Bergman *et al.*, 2013). This profile of dopamine receptor antagonism may have reduced stepping at later time points when the ratio of buspirone to its metabolites was decreased.

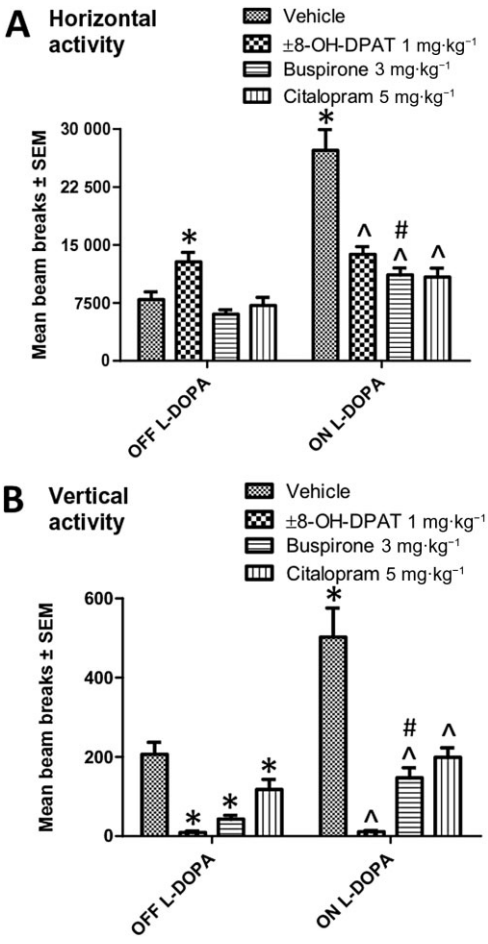

Figure 5

Activity in locomotor chambers after a combination of 5-HT compounds and L-DOPA. Rats (n = 22) were injected with ±8-OH-DPAT (1 mg·kg^{-1}), buspirone (3 mg·kg^{-1}), citalopram (5 mg·kg^{-1}) or their vehicle. Fifteen minutes later, they received L-DOPA (6 mg·kg^{-1}) or vehicle and were immediately placed in the chambers where their behaviour was recorded continuously for 180 min. (A) Number of infrared beam breaks along the horizontal plane. (B) Number of beam breaks along the vertical plane. *P < 0.01 versus vehicle OFF L-DOPA; ∧P < 0.01 versus vehicle ON L-DOPA; #P < 0.01 versus buspirone 3 mg·kg^{-1} OFF L-DOPA.

While the 5-HT compounds appeared to enhance forelimb motor performance, effects on spontaneous motor activity were mixed. Horizontal activity was increased after ±8-OH-DPAT administration while vertical activity was reduced by all three drugs (±8-OH-DPAT > buspirone > citalopram; Figure 5). This is in accord with previous work in Sprague-Dawley rats showing that ±8-OH-DPAT increased ambulation at doses between 0.06 and 2.0 mg·kg^{-1} (Tricklebank *et al.*, 1984), but it contrasts with work in Wistar rats showing horizontal and vertical activity reductions at doses of 0.05–1.6 mg·kg^{-1} (Hillegaart *et al.*, 1989). Features of

5-HT syndrome behaviours may have accounted for our observed effects. Increased horizontal activity by ±8-OH-DPAT corresponded with the time course of reciprocal forepaw treading and muscular spasticity. Decreased vertical activity with ±8-OH-DPAT and buspirone likely reflected flat body posture, but may also have indicated a reduction in exploratory rearing due to increased PD symptoms.

Interaction of L-DOPA and 5-HT syndrome with motor performance and motor activity

When combined with L-DOPA, each compound provided additional reductions of PD akinesia. While previous research has established that 5-HT_{1A} agonists and SSRIs do not impair L-DOPA efficacy at peak L-DOPA plasma levels (~45–60 min post-injection: Dekundy *et al.*, 2007; Eskow *et al.*, 2007; Conti *et al.*, 2014), for the first time, we investigated if these 5-HT drugs affected motor performance while L-DOPA efficacy was waxing or waning (30 and 120 min post L-DOPA). While L-DOPA alone did not improve stepping 30 min after injection, the addition of ±8-OH-DPAT, buspirone or citalopram increased stepping compared with vehicle (Figure 4B). At the later time point when L-DOPA was effective at augmenting stepping (120 min post L-DOPA and 135 min post 5-HT drug), ±8-OH-DPAT potentiated this increase (Figure 4B). On the intact side, compared with L-DOPA alone, ±8-OH-DPAT increased stepping while buspirone decreased stepping (Figure 4A). With the arguable exception of buspirone, this strongly suggests that 5-HT syndrome in the hemiparkinsonian rat does not interfere with the aspects of motor performance we have measured in the present study. At the same time, since each 5-HT drug increased stepping as monotherapy and as co-therapy with L-DOPA (Figures 3B and 4B), our data suggest that modifications to motor performance brought about by combining 5-HT_{1A} agonists or SSRIs with L-DOPA may be additive rather than synergistic.

Spontaneous motor activity in the horizontal and vertical planes was increased several fold by L-DOPA and was tempered by co-administration of each 5-HT compound (Figure 5). As others have noted, interpretation of motion chambers data is difficult when animals are given a dyskinesiogenic agent such as L-DOPA as the movements recorded reflect a combination of voluntary and involuntary movements (Bezard *et al.*, 2013). Compared with L-DOPA alone, the addition of each 5-HT drug decreased both horizontal and vertical activity. These decreases likely reflect LID suppression rather than L-DOPA efficacy suppression as rats were pre-exposed to the chambers and testing was performed during their daylight (inactive) period, both of which are factors that minimize exploratory behaviour.

Clinical implications

When administering 5-HT compounds to PD patients, PD-related side effects are often considered as several studies have suggested that 5-HT_{1A} agonists increase PD disability and/or attenuate L-DOPA-mediated reductions in PD symptoms (Iravani *et al.*, 2006; Dekundy *et al.*, 2007; Gregoire *et al.*, 2009). The present research suggests that 5-HT syndrome should be considered as a more common side effect. In our study, high doses of the 5-HT_{1A} agonists buspirone and ±8-OH-DPAT reduced LID by 80% and there was no evidence for increased PD akinesia (although spontaneous activity was altered). However, one side effect did coincide with LID suppression even with the low doses of 5-HT_{1A} agonists: 5-HT syndrome. While there are few clinical reports of 5-HT syndrome in the PD literature, the symptoms are almost certainly underreported: for example, 85% of English doctors who prescribed nefazodone – a drug known to produce 5-HT syndrome – were unaware that 5-HT syndrome existed as a diagnosis (Mackay *et al.*, 1999).

The results of the present research raise several key clinical questions that future studies should address. It is unclear if L-DOPA potentiates 5-HT syndrome induction by a 5-HT_{1A} agonist or SSRI, similar to what is seen when L-DOPA is co-administered with a MAO inhibitor or bromocriptine (Jacobs, 1974; Deakin and Dashwood, 1981; Sandyk, 1986). Also, given the fact that L-DOPA exposure has plastic effects on the 5-HT system (e.g. Rylander *et al.*, 2010), it is possible that chronic L-DOPA treatment alters PD patients' susceptibility to 5-HT syndrome.

In our study, the fact that citalopram suppressed LID without producing 5-HT syndrome liability indicates its potential clinical utility. However, an important caveat is that our measurements of 5-HT syndrome are putatively 5-HT_{1A} receptor-mediated symptoms (Lucki, 1992; Haberzettl *et al.*, 2013). The clinical manifestation of 5-HT syndrome includes hyperthermia and cognitive symptoms mediated by 5-HT_{2A} receptors (Boyer and Shannon, 2005). Thus, an SSRI like citalopram may produce 5-HT_{2A}-mediated features of 5-HT syndrome that were not assessed in the present experiment.

In PD patients, targeting 5-HT function remains strategically justified as the 5-HT system is involved in the pathophysiology and treatment of PD (Carta *et al.*, 2007; Huot *et al.*, 2011b; Loane *et al.*, 2013). Our research suggests that future clinical trials using 5-HT pharmacotherapies in the treatment of motor and non-motor PD symptoms should additionally incorporate 5-HT syndrome as an outcome measurement.

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Author contributions

D. L. and C. B. designed the experiments. D. L., N. P., C. Y. O., N. V. and M. M. C. performed the experiments. D. L. and C. B. analysed the data. D. L. and C. B. wrote the paper. C. B. provided funding.

Conflict of interest

None.

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