Serotonin 1B receptor stimulation reduces D1 receptor agonist-induced dyskinesia
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Dopamine replacement therapy for the treatment of Parkinson's disease leads to deleterious abnormal involuntary movements (AIMs), known as L-3,4-dihydroxyphenylalanine (L-DOPA)-induced dyskinesia, which parallels enhanced striatal dopamine D1 receptor-mediated signaling. Recent evidence suggests stimulation of striatal serotonin (5-HT) 1B receptors may reduce D1-mediated signaling. Given this potential antidykinetic mechanism, male hemiparkinsonian Sprague–Dawley rats received treatments of D1 receptor agonist, SKF81297, (0.8 mg/kg) or L-DOPA (12 mg/kg, subcutaneous injection). Dyskinetic movements were rated using the AIMs scale. Rats were then administered vehicle (100% dimethyl sulfoxide) or the 5-HT1B receptor agonist, CP94253, (1.5 or 3.0 mg/kg, subcutaneous injection), followed by SKF81297 or L-DOPA and rated for AIMs. Results indicate that CP94253 mitigates both L-DOPA and D1 receptor agonist-induced dyskinesia. These findings suggest that 5-HT1B receptor stimulation directly diminishes D1 receptor-mediated dyskinesia, implicating an important target for the treatment of L-DOPA-induced dyskinesia. NeuroReport 00:000–000 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Introduction
Dopamine (DA) replacement therapy with L-3,4-dihydroxyphenylalanine (L-DOPA), initially improves the primary symptoms of Parkinson’s disease, including akinesia, bradykinesia, and postural instability. However, adverse side effects develop after prolonged treatment, including abnormal involuntary movements (AIMs) of the extremities, face and trunk, termed L-DOPA-induced dyskinesia (LID).

Serotonin (5-HT) neuroplasticity is likely to play a major role in LID. In fact, in DA depleted rats, serotonergic neurons of the raphe nuclei convert L-DOPA into DA and release it into the striatum in an unregulated manner [1–3]. A similar event may account for LID in Parkinson’s disease, and this is supported in part by the antidykinetic effects of 5-HT1 agonists that reduce raphe activity [4–7]. Although the mechanisms underlying the effects of 5-HT1A receptor agonists have been investigated [8–11], those responsible for 5-HT1B receptor agonist effects remain speculative. Previous studies have shown that the 5-HT1B/D agonist SKF-99101-H [12] and the selective 5-HT1B receptor agonist, CP942153 [13] significantly reduced LID in DA-depleted monkeys.

Although 5-HT1B receptor agonists may provide antidykinetic action through autoregulation of L-DOPA-derived DA release, Zhang et al. [14] recently observed that striatal 5-HT1B receptor mRNA and protein were upregulated following L-DOPA treatment. This upregulation was blocked by coadministration of the D1 receptor antagonist SCH23390, suggesting an interplay between striatal D1 receptors and the effects of 5-HT1B receptor agonists. Despite this assertion, direct pharmacological evidence for D1 and 5-HT1B receptor interaction is needed. Thus, we tested the influence of 5-HT1B receptor stimulation on D1 receptor agonist-dyskinesia or LID and motor performance.

Materials and methods
Animals
Adult male Sprague–Dawley rats (225–250 g; Taconic Farms, Hudson, New York, USA) were housed in plastic cages (22 cm high, 45 cm deep and 23 cm wide) with free access to standard lab chow (Rodent Diet 5001; Lab Diet, Brentwood, Missouri, USA) and water. The colony was maintained on a 12:12 h light:dark cycle (lights on at 07:00 h) at a temperature of 22–23°C. Animals were maintained in accordance with the guidelines of the Institutional Animal Care and Use Committee of Binghamton University and the ‘Guide for the Care and Use of Laboratory Animals’ (Institute of Laboratory Animal Resources, National Academic Press 1996; NIH publication number 85–23, revised 1996).
6-hydroxydopamine lesion surgeries
One week after arrival, rats received unilateral 6-hydroxydopamine (6-OHDA) lesions of the left medial forebrain bundle (anterior/posterior: −1.8 mm, medial/lateral: +2.0 mm, dorsal/ventral: −8.6 mm relative to bregma with the incisor bar positioned 3.3 mm below the interaural line) to destroy DA neurons [8–10,15]. 6-OHDA (12 μg; Sigma-Aldrich, St. Louis, Missouri, USA) dissolved in 0.9% NaCl + 0.1% ascorbic acid was infused at a rate of 2 μl/min for a total volume of 4 μl. The needle was withdrawn 5 min later and rats were placed in clean cages on warming pads to recover from the surgery, after which they were returned to group-housing (two rats/cage).

Pharmacological treatments
To test the effects of 5-HT1B stimulation on dyskinesia, rats in the first study received L-DOPA methyl ester (1-L-DOPA; 12 mg/kg, subcutaneous injection (s.c.); Sigma-Aldrich) + DL-serine 2-(2,3,4-trihydroxybenzyl) hydrazide hydrochloride (benserazide; 15 mg/kg, s.c.; Sigma-Aldrich) once daily for 7 days (n = 10) [7]. Animals not developing moderate AIMs were eliminated from the study (total AIMs ≤ 30 on day 7). L-DOPA and benserazide were dissolved in Vehicle (VEH, 0.9% NaCl containing 0.1% ascorbic acid) and administered at a volume of 1.0 ml/kg. Every 3–4 days after priming, in a within-subjects design rats received VEH (100% dimethyl sulfoxide) or various doses of the 5-HT1B agonist 5-propoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-pyrrolo[3,2-b]pyridine hydrochloride (CP94253, 1.5 or 3.0 mg/kg, s.c.; Tocris Bioscience, Ellisville, Missouri, USA) 5 min before the injection of L-DOPA (12 mg/kg, + benserazide 15 mg/kg, s.c.). Immediately after the treatments, rats were monitored for AIMs and rotations for 2.5 h.

A second group of 6-OHDA-lesioned rats (n = 9) was used to determine whether CP94253 hinders the effects of L-DOPA on motor performance with the forepaw adjusting steps (FAS) test. L-DOPA (12 mg/kg + benserazide, 15 mg/kg, s.c.) was administered for 7 days to induce stable AIMs. Every 2–4 days after priming, rats were given one of the following treatment regimens in a within-subjects design: VEH + VEH, VEH + L-DOPA (4 mg/kg + benserazide, 15 mg/kg, s.c.), CP94253 (3 mg/kg, s.c.) + VEH, CP94253 (3 mg/kg) + L-DOPA (4 mg/kg + benserazide, 15 mg/kg, s.c.). The lower dose of L-DOPA was used to ensure that severe forelimb dyskinesia would not impair the ability of the rats to perform the task. One hour after the treatment, the FAS test was executed.

Using a procedure that we have previously used to elicit stable AIMs and rotations in 6-OHDA-lesioned rats [9,10], a separate group of rats (n = 13) received the D1 receptor agonist, SKF81297 (0.8 mg/kg, s.c.; Sigma-Aldrich) for 3 days over a 7-day period. Animals that did not develop moderate AIMs were eliminated from the study (total AIMs ≤ 30 on day 7). To determine the effects of 5-HT1B stimulation on SKF81297-mediated dyskinesia, rats were pretreated with VEH (100% dimethyl sulfoxide) or CP94253 (1.5 or 3.0 mg/kg, s.c.), as above. Five minutes later, animals were injected with SKF81297 (0.8 mg/kg, s.c.) and subsequently monitored for AIMs and rotations over a 2.5 h period.

Abnormal involuntary movements
Rats were monitored for AIMs using a procedure similar to that described previously [16,17]. On test days (09:00–14:00 h), rats were individually placed in plastic trays 5 min before the drug treatments. Following injections, each rat was assessed for exhibition of axial, limb, and oro/lingual movements. At 10 min intervals (i.e. 10, 20, 30, 40 min, etc), AIMs were rated for 60 s for each rat for a total of 2.5 h (180 min). During which a severity score of 0–4 was assigned for each AIMs category. For each AIMs category, the scores were summed for each time point.

Forepaw adjusting steps
The number of adjusting steps taken by the forelimb to compensate for movement were counted to determine the effects of CP94253 on motor performance [7,8,10]. Rats were moved across a table at a steady rate of 90 cm/10 s. The rear torso and hindlimbs were lifted from the table and one forepaw was held by the experimenter so as to bear weight on the other forepaw. Data was derived by summing forehand and backhand steps of the lesioned forelimb and dividing them by the sum of steps of the intact forelimb and multiplying by 100. This calculation yields a percentage of the intact side indicating the degree of forepaw disability.

Data analyses
Nonparametric repeated measures Friedman tests determined the treatment effects for AIMs. Significant differences between treatments were examined by Wilcoxon matched pairs post-hocs. One-way analysis of variances and least significant differences post-hoc tests were employed for analyses of rotations and FAS. α was set at P value less than 0.05. Statistical analyses were conducted with Statistica Software version 98 (Statsoft, Inc., Tulsa, Oklahoma, USA).

Results
Effects of serotonin 1B receptor stimulation on L-DOPA-induced abnormal involuntary movements and rotations
Two doses of CP94253 were tested in L-DOPA-primed rats to determine their effects on AIMs and rotations. Significant pretreatment effects were observed on axial/limb/orolingual AIMs at the 30th (χ² = 6.06), 40th
(χ² = 6.06), 50th (χ² = 7.47), 70th (χ² = 8.38), and 110th min (χ² = 7.41; all \( P < 0.05 \); Fig. 1a). Post-hoc analyses at the 40th and 50th min time points revealed that the 3 mg/kg dose of CP94253 diminished AIMs compared with VEH (all \( P < 0.05 \)). The 1.5 mg/kg dose of CP94253 also alleviated L-DOPA-induced AIMs at the 50th min time point, while enhancing AIMs at the 70th and 110th min compared with VEH pretreatment (all \( P < 0.05 \)). At the 40th min after the L-DOPA treatment, a dose-dependent reduction in AIMs was observed in CP94253 treated rats (\( P < 0.05 \)).

As shown in Fig. 1b, there was a significant main effect of time on L-DOPA-induced rotations [\( F(14,112) = 11.202; P < 0.05 \)]. There were no treatment effects or treatment by time interactions.

**Effects of serotonin 1B receptor stimulation on motor performance**

Based on the results from the first experiment, the FAS test was used to determine whether the highest dose of CP94253 (3 mg/kg) affects L-DOPA’s efficacy (Fig. 2). One-way analysis of variance indicated a significant effect of the treatment (\( F(3,24) = 6.17; P < 0.05 \)). Post-hoc analyses revealed that only coadministration of CP94253 + L-DOPA significantly improved stepping of the lesioned forepaw to 67% of intact forepaw stepping (\( P < 0.05 \)). There were no significant treatment effects of CP94253 on intact forepaw stepping.

**Effects of serotonin 1B receptor stimulation on D1 receptor agonist-induced abnormal involuntary movements and rotations**

To evaluate a potential interaction between 5-HT1B and D1 receptors, the effects of two doses of CP94253 on AIMs and rotations were tested in SKF81297-primed rats. As shown in Fig. 3a, time course analysis of axial/limb/orolingual AIMs revealed significant effects of pretreatment with both doses of CP94253 at the 40th (\( \chi^2 = 7.94 \)), 50th (\( \chi^2 = 8.05 \)), 60th (\( \chi^2 = 7.74 \)), 70th (\( \chi^2 = 9.80 \)) and 120th (\( \chi^2 = 8.34 \)) min time points after SKF81297 treatment (all \( P < 0.05 \)). Post-hoc analyses at these time points showed a significant reduction in AIMs with the 3 mg/kg dose of CP94253 at the 40–70th min (all \( P < 0.05 \)). The 1.5 mg/kg dose of CP94253 decreased AIMs at the 40th min, whereas increasing AIMs at one time point (120th min; both \( P < 0.05 \)).

Although a significant main effect of time [\( F(14,112) = 11.202; P < 0.05 \)] was observed upon
inspection of rotations, there was no effect of treatment or a treatment by time interaction (Fig. 3b).

Discussion
These results show that selective 5-HT1B receptor stimulation with CP94253 alleviates LID in the hemiparkinsonian rat, while improving motor performance. More importantly, we provide novel evidence that D1 receptor-mediated dyskinesia is similarly counteracted by CP94253, indicating that the antidysskineic action of 5-HT1B receptor stimulation occurs in part through a mechanism that involves modulation of D1 receptor function.

Previous investigations into the antidyskineic effects of 5-HT1B receptor agonists in LID have emphasized a potential regulatory role, whereby 5-HT1B receptor stimulation curtails excessive DA release from 5-HT neurons. For example, Carta et al. [3] found that subthreshold doses of CP94253 had a synergistic antidyskineic effect when administered in conjunction with the 5-HT1A receptor agonist ± 8-hydroxy-2-(di-n-propylamino)-tetralin. Our results corroborate that 5-HT1B receptor agonists hold potential as a treatment for LID, as CP94253 effectively reduced AIMS in l-DOPA-primed, hemiparkinsonian rats and improved motor performance on the FAS test when given as an adjunct with l-DOPA.

In LID, striatal D1 receptors appear to be particularly important given their markedly increased expression and signaling in dyskinetic animals and humans [18,19]. Indeed, coadministration of a D1 receptor antagonist with l-DOPA can block LID development [20]. In this study, D1 receptor agonist-induced dyskinesia was counteracted by administration of CP94253. Thus, the mechanism underlying its antidyskineic effects is likely to be not mediated solely by its actions as a serotonergic autoreceptor. Although 5-HT1B receptors are only found at low levels in the raphe nuclei, pronounced 5-HT1B receptor densities in the substantia nigra and mRNA transcript in the striatum has been reported [21]. This suggests that 5-HT1B receptors may act as presynaptic heteroreceptors within striatal output pathways projecting to the substantia nigra and globus pallidus. Previous research indicates that stimulation of 5-HT1B receptors can temper the release of γ-aminobutyric acid from striatonigral projections in the D1 receptor output pathway [22], potentially mitigating the overactivity of this pathway, thought to contribute to the expression of LID [18,23].

In support, Zhang et al. [14] showed an antidyskineic effect of CP94253 on l-DOPA-induced AIMS in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned mice. In mice expressing a knockout of a key regulator protein for the 5-HT1B receptor, p11, the antidyskineic effect of 5-HT1B receptor stimulation was curtailed. Interestingly, mRNA and protein expression of 5-HT1B receptor was upregulated after the L-DOPA administration in parkinsonian rats and mice. These effects were blocked by administration of a D1 receptor antagonist SCH23390, suggesting that the striatonigral pathway is directly involved in the changes observed in 5-HT1B receptor expression observed upon L-DOPA treatment.

Traditionally, rotational behavior induced by dopamine agonists and l-DOPA is considered to be indicative of a drug’s antiparkinsonian efficacy [24]. In this study, CP94253 had no effect on rotations induced by either l-DOPA or SKF81297 and actually improved stepping, suggesting that 5-HT1B receptor agonists may improve motor disability when given in conjunction with l-DOPA.
Conclusion
The selective 5-HT1B receptor agonist, CP94 253 exerted antidyskinetic effects in L-DOPA-primed hemiparkinsonian rats while improving motor performance in this study. More importantly, it held similar antidyskinetic action in D1 receptor agonist-primed rats. The mechanism underlying its potential utility for LID treatment may be based upon its interaction with D1 receptors, likely serving to temper excessive D1-mediated g-aminobutyric acid release from striatonigral projection neurons.

Acknowledgements
This study was supported by funds from the Center for Development and Behavioral Neuroscience at Binghamton University (C.B.) and NIH NS059600 (C.B.).

References