Local modulation of striatal glutamate efflux by serotonin 1A receptor stimulation in
dyskinetic, hemiparkinsonian rats

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Abstract
Serotonin 1A receptor (5-HT₁₆R) agonists reduce both L-DOPA- and D1 receptor (D1R) agonist-mediated dyskinesia, but their anti-dyskinetic mechanism of action is not fully understood. Given that 5-HT₁₆R stimulation reduces glutamatergic neurotransmission in the dopamine-depleted striatum, 5-HT₁₆R agonists may diminish dyskinesia in part through modulation of pro-dyskinetic striatal glutamate levels. To test this, rats with unilateral medial forebrain bundle dopamine or sham lesions were primed with L-DOPA (12 mg/kg + benserazide, 15 mg/kg, sc) or the D1R agonist SKF81297 (0.8 mg/kg, sc) until abnormal involuntary movements (AIMs) stabilized. On subsequent test days, rats were treated with vehicle or the 5-HT₁₆R agonist ±8-OH-DPAT (1.0 mg/kg, sc), followed by L-DOPA or SKF81297, or intrastriatal ±8-OH-DPAT, or intrastriatal ±8-OH-DPAT (7.5 or 15 mM), followed by L-DOPA. In some cases, the 5-HT₁₆R antagonist WAY100635 was employed to determine receptor-specific effects. In vivo microdialysis was used to collect striatal samples for analysis of extracellular glutamate levels during AIMs assessment. Systemic and striatal ±8-OH-DPAT attenuated L-DOPA-induced dyskinesia and striatal glutamate efflux while WAY100635 reversed ±8-OH-DPAT's effects. Interestingly, systemic ±8-OH-DPAT diminished D1R-mediated AIMs without affecting glutamate. These findings indicate a novel anti-dyskinetic mechanism of action for 5-HT₁₆R agonists with implications for the improved treatment of Parkinson's disease.

Introduction
Chronic dopamine (DA) replacement therapy with L-3,4-dihydroxyphenylalanine (L-DOPA) for Parkinson's disease (PD) patients often results in abnormal and excessive movements known as L-DOPA-induced dyskinesia (LID; Jankovic, 2005). Although the mechanisms of LID are not fully understood, it is believed that following DA depletion, serotonergic neurons of the raphe nuclei convert exogenously administered L-DOPA to DA and release it into the striatum in a pulsatile, unregulated manner (Carta et al., 2007; Eskow et al., 2009; Lindgren et al., 2010). This aberrant release of DA is believed to stimulate supersensitive DA D1 (D1R) and D2 (D2R) receptors located in the DA-depleted striatum (Pavese et al., 2006; Cenci, 2007). While both receptor subtypes appear to be involved in LID, it is likely that striatal D1R have a more prominent role (Westin et al., 2007). For instance, striatal D1R expression and signaling have been shown to be significantly enhanced in dyskinetic animals and humans (Cenci et al., 1998; Gerfen et al., 2002; Aubert et al., 2005; Guigoni et al., 2007), and D1R agonists induce dyskinesia in both experimental and clinical models of PD (Rascoc et al., 2001; 2006; Delfino et al., 2007; Dupre et al., 2007, 2008a).

It is well known that serotonin (5-HT) 1A receptor (5-HT₁₆R) agonists diminish LID (Dekundy et al., 2007; Eskow et al., 2007, 2009) and these results have been mostly attributed to stimulation of raphe 5-HT₁₆R that temper striatal DA release. Interestingly, there is also evidence that stimulation of 5-HT₁₆R located directly within the striatum attenuates both L-DOPA- (Bishop et al., 2009) and D1R-mediated dyskinesia (Dupre et al., 2008a) and improves movement in DA-depleted rats (Mignon & Wolf, 2002; Matsubara et al., 2006; Dupre et al., 2008a). The mechanism(s) surrounding these striatally-
mediated 5-HT1AR effects are not yet known. One possibility is that activation of these receptors, located presynaptically on corticostriatal glutamate neurons, attenuate the release of glutamate into the striatum (Antonacci et al., 2005; Mignon & Wolf, 2005). Indeed, upon DA depletion and subsequent i-DOPA or D1R agonist treatment, augmentation of striatal glutamate levels (Jonkers et al., 2002; Robelet et al., 2004) and increased expression of striatal glutamate receptors have been postulated to result in dyskinetic behaviors (Calon et al., 1992; Ferré et al., 1994; Bibbiani et al., 2005; Rylander et al., 2010; Kobylecki et al., 2010). Thus, whether striatal 5-HT1AR stimulation attenuates LID and D1R agonist-induced dyskinesia through modulation of local glutamate release remains an important mechanistic and translational question.

The aim of the current study was to investigate the effects of systemic and local 5-HT1AR stimulation on extracellular striatal glutamate levels in hemiparkinsonian rats rendered dyskinetic by either i-DOPA or the D1R agonist SKF81297. Using in vivo microdialysis, the full 5-HT1AR agonist (±)-8-Hydroxy-2-(dipropylamino)tetrailinhydrobromide (±8-OH-DPAT) was administered systemically or striatally perfused prior to i-DOPA (12 mg/kg, sc + benserazide, 15 mg/kg, sc) or administered systemically to R(+)-SKF-81297 hydrobromide (SKF81297: 0.8 mg/kg, sc) in unilaterally DA-depleted and sham-lesioned rats. Striatal sample fractions were collected for analysis of glutamate and dyskinesia was measured using the abnormal involuntary movements (AIMs) scale (Lundblad et al., 2002). The present results indicate that the anti-dyskinetic effect of 5-HT1AR stimulation coincides with a reduction in extracellular striatal glutamate levels with i-DOPA, but not D1R agonist, treatment. These findings implicate a novel glutamatergic mechanism by which 5-HT1AR agonists work to reduce LID with implications for the treatment of PD.

Materials and methods

Animals

Adult male Sprague–Dawley rats (N=61) were used (225–250 g upon arrival; Taconic Farms, Hudson, NY, USA). Rats were kept in plastic cages (22 cm high, 45 cm deep and 23 cm wide) and given free access to food (Rodent Diet 5001; Lab Diet, Brentwood, MO, USA) and water. The colony room was kept on a 12 h light/dark cycle (light on at 0700 h) and maintained at 22–23°C. 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) were maintained throughout the study.

Medial forebrain bundle 6-hydroxydopamine lesion and microdialysis guide cannulae implantation surgeries

One week after arrival, rats in Experiments 1 and 2 received unilateral DA (n=24) or sham (n=18) lesions of the left medial forebrain bundle (MBF). All rats in Experiment 3 received DA lesions of the left MBF (n=20). Each rat was administered desipramine HCl (25 mg/kg, ip; Sigma, St. Louis, MO, USA) 30 min prior to surgery in order to protect norepinephrine neurons. Rats were anesthetized with inhalant isoflurane (2–3%; Sigma) in oxygen (2.5 L/min) and placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). The following coordinates relative to bregma were used for the site of injection: AP, −1.8 mm; ML, +2.0 mm; DV, −8.6 mm, with the incisor bar positioned at 5.0 mm below interaural length (Paxinos & Watson, 1998). After drilling a small hole in the skull above the site of injection, a 10 μl Hamilton syringe attached to a 26 gauge needle was lowered into the target. At that point, 4 μl of vehicle (0.90% sodium chloride (NaCl) + 0.1% ascorbic acid) or 6-hydroxydopamine hydrobromide (6-OHDA; 3 μg/μl; Sigma) was injected at a rate of 2 μl/min for rats in Experiments 1 and 2, whereas all rats in Experiment 3 received 6-OHDA. The needle was withdrawn 5 min later. During the same surgery, rats were fitted unilaterally with plastic microdialysis guide cannulae (CMA 12 Elite; Stockholm, Sweden) targeting the dorsal striatum ipsilateral to the lesioned side (AP, +1.2 mm; ML, +2.5 mm; DV, −3.7 mm; relative to bregma; Paxinos & Watson, 1998). Cannulae were positioned and affixed to the skull with screws and liquid and powder dental acrylic (Lang Dental, Wheeling, IL). At the completion of surgery, animals were single housed, placed in clean cages and allowed to recover with ad lib food and water. Five minute pre-surgery and 1 h and 1 day post-surgery, rats received an injection of Buprenex (buprenorphine HCl; 0.03 mg/kg, ip; Reckitt Benckiser Pharmaceuticals Inc., Richmond, VA) as analgesic treatment. Soft chew was also provided and rats were monitored and handled twice per week for 3 weeks post-surgery in order to ensure full recovery and acclimation to experimenters.

Pharmacological treatments and in vivo microdialysis procedure

Experiment 1: effects of systemic 5-HT1AR stimulation on extracellular striatal glutamate levels in i-DOPA-induced dyskinesia

Three weeks after 6-OHDA (n=14) or sham (n=11) lesions of the MFB and unilateral striatal microdialysis cannulations, rats in the first experiment received injections of l-3,4-dihydroxyphenylalanine methyl ester hydrochloride (l-DOPA; 12 mg/kg, sc; Sigma) + dl-serine 2-(2,3,4-trihydroxybenzyl) hydrazide hydrochloride (benserazide; 15 mg/kg, sc; Sigma) once daily for 7 days. The dose of l-DOPA and the length of priming have been extensively utilized in our lab to produce prominent and stable AIMs expression (Eskow et al., 2007; Dupre et al., 2008b; Bishop et al., 2009). l-DOPA and benserazide were dissolved in vehicle (0.9% NaCl + 0.1% ascorbic acid). On the final day of priming, AIMs (see description below) were observed every 20 min for 3 h immediately after l-DOPA injections. 6-OHDA-lesioned rats displaying an AIMs score of ≥25 by the 7th day of l-DOPA priming were retained for further study (n=12).

Microdialysis testing commenced 2 days after the last day of l-DOPA priming. On test day, striatal probes (CMA 12 Elite; membrane length = 3 mm; 20 000 Dalton; Stockholm, Sweden) were inserted into rats’ guide cannulae and locked into place so that the dialysis membrane extended −3.7 to −6.7 ventral to bregma within the striatum. After 60 min of probe stabilization (2.0 μl/min of artificial cerebral spinal fluid (aCSF) (in mM: NaCl, 125; KCl, 1.3; CaCl2, 2.1; MgCl2, 0.9; NaH2PO4, 2.0; NaHPO4, 1.0 glucose, brought to a pH of 7.4)), striatal dialysate samples were collected every 20 min for 40 min to determine baseline levels of glutamate. At this point, rats received a systemic treatment injection of vehicle (0.9% NaCl + 0.1% ascorbic acid, sc) and sample fractions were collected every 20 min for 2 h to determine any changes in extracellular glutamate levels due to systemic injection. Following this, using a counter-balanced design, rats received systemic treatment of: Vehicle (0.9% NaCl), the full 5-HT1AR agonist ± 8-OH-DPAT (1.0 mg/kg, sc; Sigma), or combined ± 8-OH-DPAT (1.0 mg/kg, sc) + the 5-HT1AR antagonist N-[2-(2-Methoxyphenyl)-1-piperazinyl][ethyl]-N-2-pyridylindolocyclohexanecarboxamide maleate salt (WAY100635; 0.5 mg/kg, sc; Sigma), immediately followed by l-DOPA (12 mg/kg, + benserazide, 15 mg/kg, sc). Sample fractions were collected every 20 min for 3 h and AIMs were concurrently observed during this time. Each rat underwent this microdialysis procedure for 2 consecutive days. No differences in glutamate nor AIMs were found in animals treated with Vehicle + l-DOPA on microdialysis test day 1 versus test day 2 (data not shown).
In addition, drug clearance of ±8-OH-DPAT by day 2 of testing was ensured due to its relatively short half-life of approximately 45 min (Yu & Lewander, 1997). Finally, a post-test with i-DOPA alone was performed at the end of the study to ensure stable AIMs throughout testing (Fig. 1).

Experiment 2: effects of systemic 5-HT<sub>1A</sub>R stimulation on extracellular striatal glutamate levels in D1R agonist-mediated dyskinesia

Three weeks after 6-OHDA (n = 10) or sham (n = 7) lesions of the MFB and unilateral striatal microdialysis cannulations, rats in the second experiment received injections of the D1R agonist SKF81297 (0.8 mg/kg, sc; Sigma), dissolved in 2% dimethyl sulfoxide (DMSO) in 0.9% NaCl on 3 separate occasions 2–3 days apart in order to sensitize D1R (Pollack & Yates, 1999; Dupre et al., 2007). The dose of SKF81297 and priming regimen have been used in our lab to produce stable AIMs expression that is similar to the AIMs induced by our current dose of i-DOPA (Dupre et al., 2007; Dupre et al., 2008a). AIMs were observed every 20 min for 3 h immediately after injections. 6-OHDA-lesioned rats displaying an AIMs score of ≥25 by the 3rd day of D1R priming were retained for further study (n = 9). Microdialysis testing commenced 2 days after the last day of SKF81297 priming.

Rats in Experiment 2 followed a similar microdialysis procedure as those in Experiment 1. Following baseline sampling, rats received a systemic treatment injection of vehicle (20% DMSO in 0.9% NaCl, sc) and sample fractions were collected every 20 min for 2 h. At this point, using a counter-balanced, within-subjects design, rats received systemic treatment of vehicle (0.9% NaCl or ±8-OH-DPAT (1.0 mg/kg, sc) immediately followed by SKF81297 (0.8 mg/kg, sc). Sample fractions were collected every 20 min for 3 h and AIMs were concurrently observed during this time. Each rat underwent this microdialysis procedure for 2 consecutive days and no differences in glutamate nor AIMs were found in animals treated with Vehicle + SKF81297 on microdialysis test day 1 versus test day 2 (data not shown). A post-test with SKF81297 alone was performed at the end of the study to ensure that there were stable AIMs throughout the testing (Fig. 1).

Experiment 3: Effects of intrastriatal 5-HT<sub>1A</sub>R stimulation on local extracellular glutamate levels in i-DOPA-induced dyskinesia

Three weeks after 6-OHDA lesions of the MFB and unilateral striatal microdialysis cannulations, rats in the third experiment received injections of i-DOPA (12 mg/kg, benzerazide, 15 mg/kg, sc) once daily for 7 days. On the final day of priming, AIMs were observed every 20 min for 3 h immediately after i-DOPA injections. Rats displaying an AIMs score of ≥25 by the 7th day of i-DOPA priming were retained for further study (n = 18).

Microdialysis testing commenced 2 days after the last day of i-DOPA priming and followed a similar procedure as that described in Experiment 1. After baseline sampling, rats received a systemic treatment injection of vehicle (0.9% NaCl + 0.1% ascorbic acid, sc) and sample fractions were collected every 20 min for 2 h. Following this, using a counter-balanced design, rats received intrastriatal infusion of: Vehicle (aCSF), the full 5-HT<sub>1A</sub>R agonist ±8-OH-DPAT (7.5 or 15 mM), or combined ±8-OH-DPAT (15 mM)+WAY100635 (4.6 mM), followed 10 min later (when drug reached brain) by systemic treatment injections of i-DOPA (12 mg/kg, + benzerazide, 15 mg/kg, sc).
15 mg/kg, sc). Sample fractions were collected every 20 min for 3 h and AIMs were concurrently observed during this time. Each rat underwent this microdialysis procedure no more than 2 times and a post-test with i-DOPA alone was performed at the end of the study to ensure stable AIMs throughout testing (Fig. 1).

**Abnormal involuntary movements**

Rats were monitored for AIMs using a procedure similarly described in Dupre et al. (2008a; 2008b). The AIMs model of dyskinesia utilizes distinct behavioral measures and demonstrates face validity with known anti-dyskinetic compounds (Lundblad et al., 2002; Dekundy et al., 2007). AIMs can also be maintained over repeated testing by separating experimental days after initial priming (Bishop et al., 2006; Taylor et al., 2006). On the last day of priming (0900−1700 h), rats were individually placed in clear plastic cylinders (20 × 25 cm) immediately following i-DOPA or SKF81297 injection. On microdialysis test days (0800−1800 h), animals received treatment injections while in clear plastic round bottom microdialysis bowls (CMA Microdialysis Inc.). Following injections, a trained observer assessed each rat for exhibition of axial, limb, and orolingual AIMs. Dystonic posturing of the neck and torso, involving positioning of the neck and torso in a twisted manner directed toward the side of the body contralateral to the lesion, were referred to as “axial” AIMs. "Limb” AIMs were defined as rapid, purposeless movements of the forelimb located on the side of the body contralateral to the lesion. "Orolingual” AIMs were composed of repetitive openings and closings of the jaw and tongue protrusions. The movements were considered abnormal as they occurred at times when the rats were not chewing or gnawing on food or other objects. Every 20th min for 3 h, rats were observed for 1 min for AIMs. During this time, a severity score of 0–4 was assigned for each AIMs category: 0, not present; 1, present for <50% of the observation period (i.e. 1−29 s); 2, present for >50% or more of the observation period (i.e. 30−59 s); 3, present for the entire observation period (i.e. 60 s) and interrupted by a loud stimulus (a tap on the cylinder), or 4, present for the entire observation period but not interrupted by a loud stimulus. Thus, the theoretical maximum score for each type of AIM was 36 (4×9 time periods), although observed scores were never this severe. The scores of the 3 AIMs subcategories (axial, limb, and orolingual) were summed (with a theoretical maximum potential score of 12 per time period (4×3 AIMs subcategories) and an overall maximum score of 108 for the entire 3 h).

**Histology and neurochemical analyses**

**Tissue dissection and cryostat sectioning**

When experiments were completed, a subset of sham-lesioned (n = 7) and 6-OHDA-lesioned (n = 7) rats were sacrificed by decapitation and brains were immediately removed. Anterior and central striata from these rats were visually examined for verification of striatal placements. To determine the level of DA depletion, posterior striata were freshly dissected, frozen at −80 °C and subjected to monoamine analysis using high performance liquid chromatography with electrochemical detection (HPLC-ED; see below). Striata from all other rats were examined for verification of striatal placements using a cryostat. During dissection, anterior and central striata were removed and rapidly frozen in methylobutane (−30 °C) and stored at −20 °C. Cresyl violet (FD Neurotechnologies, Baltimore, MD) staining was used to determine injection sites and extent of gliosis from cryostat-generated 20 mm coronal sections containing injection sites that were post-fixed with 4% paraformaldehyde (PFA; Fisher Scientific, Hanover Park, IL). All rats that completed these studies were found to have probe placements within the dorsocentral or dorsolateral aspects of the striatum (data not shown).

**High-performance liquid chromatography for DOPAC and DA tissue analyses**

Reverse-phase HPLC-ED was performed on striatal tissue, obtained from a subset of rats, according to protocol of Kilpatrick et al. (1988), a method for semiautomated catecholamine and indoleamine analysis with coulometric detection. The system included an ESA autoinjector (Model 542; Chelmsford, MA, USA), an ESA solvent delivery system (582), an external pulse dampener (ESA), an ESA column and a MD-150X3.2 (150 × 3.2 mm, 3 μm packing) column (ESA). Samples were homogenized in ice-cold perchloric acid (0.1 M) with 1% ethanol and 0.02% ethylenediaminetetraacetic acid (EDTA). The homogenates were spun for 30 min at 14,000g with the temperature maintained at 4 °C. Aliquots of supernatant were then analyzed for abundance of 3,4-dihydroxyphenylacetic acid (DOPAC) and DA. Samples were separated using a mobile phase composed of sodium phosphate (monobasic, anhydrous), 100 mM; EDTA, 0.05 mM; octane sulfonic acid, 1.4 mM; and acetonitrile, 9% adjusted to pH 3.0 with orthophosphoric acid. A coulometric detector configured with three electrodes (Coulochem III; ESA) measured the content of DOPAC and DA. An ESA model 5020 guard cell (+ 300 mV) was positioned prior to the autoinjector. The analytical cell (ESA model 5011A; first electrode at −100 mV, second electrode at + 250 mV) was located immediately after the column. The second analytical electrode emitted signals that were recorded and analyzed by EChem Elite software via Scientific Software Inc. module (SS420L). The final oxidation current values were compared to known standards 10−6−10−9, adjusted to striatal tissue weights, and expressed as nanograms (ng) of DOPAC or DA per milligram (mg) tissue (mean ± SEM).

**Dopamine tissue levels**

Dopamine tissue levels were concurrently observed during this phase of the study using a procedure described by Donzanti and Yamamoto (1988) and Rowley et al. (1995). Dopamine and DOPAC were concurrently observed during this phase of the study using a procedure described by Donzanti and Yamamoto (1988) and Rowley et al. (1995). Employed electrochemical detection of amino acid derivatives formed by a chemical reaction with o-phthalaldehyde (OPA) and β-mercaptoethanol (β-ME). The system included an ESA autoinjector (Model 542; Chelmsford, MA, USA), an external pulse dampener (ESA), and an XTierra MSC18 (3.0 × 50 mm, 2.5 μm) column. The OPA/β-ME derivatizing reagent (15 μl) reacted with striatal dialysate samples (30 μl) and 20 μl of the derivatized sample was analyzed for abundance of glutamate. The mobile phase consisted of 100 mM sodium phosphate (dibasic, anhydrous) with 20% by volume methanol and 3.5% by volume acetonitrile, filtered through 0.2 μm nylon membrane filter (Nyalop; Pall Life Sciences) and adjusted to pH 6.75 with o-phosphoric acid and/or sodium hydroxide. An ESA model 5020 guard cell (+ 700 mV) was positioned prior to the autoinjector. An analytical cell (ESA model 5014B; first electrode + 150 mV, second electrode + 400 mV) located immediately after the column was controlled by a coulometric detector configured with three electrodes (Coulochem III; ESA). The areas of oxidation current peaks generated at the second electrode by eluting amino acid derivatives was analyzed by EChem Elite software via a Scientific Software Inc. module (SS420L). The oxidation current values were converted to masses (ng of amino acid) using standard curves (2e−6−1e−3 M). The determination of probe recovery (± 30%) was performed in vitro by perfusing standard solutions (2e−6−1e−3 M).
factor repeated-measures (sample time [20 min samples over 340 min total = 17 sample times]) ANOVA. Treatment effects (expressed as means ± SEM) for striatal glutamate concentrations (M) were analyzed with between-subjects one-way ANOVAs. AIMS (expressed as medians) were analyzed by employing non-parametric Kruskal–Wallis tests. Significant differences between groups were determined by Mann–Whitney post hoc comparisons (Pett, 1997) for AIMS and planned comparison tests for striatal DOPAC, DA, and glutamate (% baseline and M). Analyses were performed with the use of Statistica software ‘98 (Statsoft Inc., Tulsa, OK, USA) and alpha was set at p < 0.05.

**Results**

### Striatal DOPAC and DA levels

Subsets of 6-OHDA- and sham-lesioned animals were examined by HPLC-ED for determination of average DopAC and DA content (see Table 1). Mixed design two-way ANOVAs [2 lesion types (between) × 2 hemispheres (within)] of striatal DOPAC and DA revealed main effects of lesion (DOPAC: F1,12 = 11.57; DA: F1,12 = 9.52; p < 0.01), hemisphere (DOPAC: F1,12 = 24.53; DA: F1,12 = 31.57; p < 0.01), and lesion × hemisphere interactions (DOPAC: F1,12 = 10.78; DA: F1,12 = 18.08; p < 0.01). Planned comparison analyses showed that unilateral 6-OHDA injection into the MFB produced significant reductions in lesioned striatal DOPAC and DA compared to the intact striatum, 98.3% and 99.7%, respectively.

Experiment 1: systemic 5-HT1AR stimulation does not alter extracellular striatal glutamate efflux while concurrently reducing AIMS in 6-OHDA-lesioned rats

The effects of systemic 5-HT1AR stimulation on striatal glutamate in DA-depleted, chronically 6-DOPA-treated rats was examined by injecting vehicle or ±8-OH-DPAT (1.0 mg/kg, sc) immediately prior to 6-DOPA treatment. A mixed design two-way ANOVA [3 treatment groups (between) × 17 time points (within)] revealed a main effect of time (F16,272 = 10.93, p < 0.01) and a treatment × time interaction (F32,272 = 3.19, p < 0.01; Fig 3A). Planned comparisons revealed that pretreatment with ±8-OH-DPAT significantly reduced striatal glutamate compared to 6-DOPA alone at time points 120–260 (all p < 0.05). Co-administration of the full 5-HT1AR antagonist WAY100635 with ±8-OH-DPAT reduced ±8-OH-DPAT’s effects at the 160th and 180th min (both p < 0.05). In sham-lesioned rats, a main effect of time for extracellular striatal glutamate levels was found (F16,256 = 50.75, p < 0.001), but there was no main effect of treatment nor a treatment × time interaction (Fig 3D). Since extracellular striatal glutamate levels lessened over sampling time in both lesion groups, main effects of time did not interfere with the treatment effects observed.

The average concentrations of striatal glutamate during 120 min of vehicle (time points 0–100) and 120 min of treatment (time points 120–220) are portrayed in Fig. 3B and E. A one-way ANOVA showed a main effect of treatment in 6-OHDA-lesioned rats (F2,205 = 12.11, p < 0.01; Fig 4B) but not sham-lesioned rats (Fig 4E). Post hoc analyses showed that 6-DOPA significantly increased striatal glutamate concentrations compared to vehicle treatment, whereas pre-treatment with ±8-OH-DPAT significantly lowered glutamate concentrations compared to all treatments in 6-OHDA-lesioned rats (all p < 0.05). Importantly, WAY100635 reversed ±8-OH-DPAT’s suppressant effects while maintaining 6-DOPA’s enhancement of striatal glutamate when compared to vehicle treatment (p < 0.05).

While there was no expression of AIMS with any treatment in sham-lesioned rats (Fig 3F), main effects for AIMS in 6-OHDA-lesioned rats were observed at the 120–240th min (all H2 > 9.0; all p < 0.05). As shown in Fig. 3C, post hoc analyses revealed that ±8-OH-DPAT significantly attenuated 6-DOPA-induced AIMS at all time points compared to 6-DOPA alone (all p < 0.05). WAY100635 successfully reversed the anti-dyskinetic effects of ±8-OH-DPAT on AIMS from the 160th–240th min (all p < 0.05).

### Table 1

**Effects of unilateral medial forebrain bundle (MFB) 6-OHDA or sham lesion on concentrations of striatal DOPAC and DA.**

<table>
<thead>
<tr>
<th>Intact vs. lesioned</th>
<th>DOPAC (ng/mg)</th>
<th>DA (ng/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subset 6-OHDA-lesioned (n = 7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact (right)</td>
<td>3.42 (± 0.16)</td>
<td>11.34 (± 1.44)</td>
</tr>
<tr>
<td>Lesioned (left)</td>
<td>0.06 (± 0.01)</td>
<td>0.03 (± 0.01)</td>
</tr>
<tr>
<td>(Lesioned/intact %)</td>
<td>1.70%</td>
<td>0.30%</td>
</tr>
<tr>
<td>Subset sham-lesioned (n = 7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact (right)</td>
<td>4.09 (± 0.74)</td>
<td>10.30 (± 1.49)</td>
</tr>
<tr>
<td>Lesioned (left)</td>
<td>3.41 (± 0.79)</td>
<td>8.74 (± 1.53)</td>
</tr>
<tr>
<td>(Lesioned/intact %)</td>
<td>88.45%</td>
<td>92.22%</td>
</tr>
</tbody>
</table>

Values are nanogram DOPAC or DA per milligram protein and percentage of intact striata. Differences between group means were determined by two-way ANOVAs and planned comparison analyses.

* p < 0.05 compared to intact sides.

+ p < 0.05 compared to lesioned side (sham).
6-OHDA- and sham-lesioned rats. Under these conditions, main effects of time were found in 6-OHDA- ($F_{1,16} = 7.50, p < 0.001$; Fig. 4A) and sham-lesioned ($F_{1,16} = 13.34, p < 0.001$; Fig. 4D) rats, but there were no main effects of treatment and no treatment × time interactions for either lesion type. Thus, similar to Experiment 1, extracellular striatal glutamate levels fell from baseline over time in both lesion groups. Next, when comparing the average striatal glutamate concentrations during the 120 min of vehicle (time points 0–100) and treatment sampling (time points 120–220), there were no significant main effects of treatment for 6-OHDA- (Fig. 4B) or sham-lesioned (Fig. 4E) rats. Therefore, neither SKF81297 nor pretreatment with ±8-OH-DPAT impacted extracellular striatal glutamate levels in 6-OHDA- and sham-lesioned animals that had been primed with SKF81297.

Although systemic 5-HT$_{1A}$R stimulation did not modify striatal glutamate in DA-depleted, ±8-OH-DPAT-treated rats, ±8-OH-DPAT robustly reduced SKF81297-induced AIMS from the 120–240th min (all $H_1 > 5.00; all p < 0.05$; Fig. 4C). In sham-lesioned rats, there was no expression of AIMS (Fig. 4F).

**Experiment 3: striatal 5-HT$_{1A}$R stimulation reduces local extracellular glutamate levels while concurrently attenuating l-DOPA-induced AIMS in 6-OHDA-lesioned rats**

Finally, the effects of striatal 5-HT$_{1A}$R stimulation on local extracellular glutamate levels in DA-depleted, ±8-OH-DPAT-treated rats were examined. Using in vivo microdialysis, aCSF or ±8-OH-DPAT (7.5 or 15 mM) was locally administered into the striatum for 2 h. A two-way ANOVA revealed a main effect of time ($F_{6,336} = 7.08, p < 0.001$) and a treatment × time interaction ($F_{48,336} = 2.10, p < 0.001$) but no main effect of treatment ($F_{2,21} = 3.39, p = 0.067$) for extracellular striatal glutamate levels (% baseline) in 6-OHDA-lesioned rats (Fig. 5A). Planned comparisons revealed that, similar to systemic ±8-OH-DPAT treatment, striatal infusion of the lower concentration of ±8-OH-DPAT (7.5 mM) significantly reduced local extracellular glutamate levels compared to ±8-OH-DPAT alone at time points 140–220 (all $p < 0.05$). The higher concentration of ±8-OH-DPAT (15 mM) also diminished striatal glutamate compared to ±8-OH-DPAT alone at the 120th, 160th, 180th, and 200th min (all $p < 0.05$). WAY100635 reversed ±8-OH-DPAT’s effects on AIMS at all time points (all $p < 0.05$).

**Discussion**

The anti-dyskinetic effects of 5-HT$_{1A}$R stimulation have been well-characterized in both preclinical (Tomiyama et al., 2005; Carta et al., 2007; Dekundy et al., 2007; Dupre et al., 2007, 2008a, 2008b; Eskow et al., 2007; Bishop et al., 2009) and clinical investigations (Bara-Jimenez et al., 2005; Goetz et al., 2007). Unfortunately, how these compounds exert their therapeutic effects is not fully understood, which has limited their use for PD patients. The current study showcases a potential mechanism by which 5-HT$_{1A}$R stimulation reduces LID by demonstrating that both systemic and intrastriatal pre-treatment with the full 5-HT$_{1A}$R agonist ±8-OH-DPAT contemporaneously diminishes striatal glutamate efflux and AIMS in l-DOPA-treated, but not D1R agonist-treated, hemiparkinsonian rats.

The loss of DA within the brain has profound, but inconsistent, effects on the corticostriatal glutamate system (Chase & Oh, 2000), such that increases (Lindelors & Unгерstedt, 1990; Meshul et al., 1999; Jonkers et al., 2002; Robinson et al., 2003), reductions (Reid et al., 1990; Meshul et al., 1999; Robinson et al., 2003), and no changes in striatal glutamate levels (Bianchi et al., 2003; Corsi et al., 2003; Robelet et al., 2004) have been reported. In the current study we found that unilateral 6-OHDA-lesions of the MFB, irrespective of the priming drug (l-DOPA or SKF81297), led to a reduction of basal levels of striatal glutamate when compared to sham-lesioned, primed rats (Fig. 3). This is similar to recent work by Morgese et al. (2009), who found in unilaterally DA-depleted, chronically l-DOPA-treated rats, basal levels of glutamate within the ipsilateral striatum were diminished compared to the contralateral striatum. Interestingly, Meshul and colleagues (1999) found that there is a time-dependent change in striatal glutamate in non-primed, DA-depleted rats, where an increase is found at 1 month post-6-OHDA-lesion but at 3 months post-lesion, a decrease is found. The early rise in striatal glutamate may be a primary response to the insult of the lesion, while the decrease that we and others report may represent a compensatory response to the initial increase.

In addition to the effects of DA denervation, chronic DA replacement therapy with l-DOPA greatly impacts the corticostriatal pathway (Ouattara et al., 2010). For example, l-DOPA administration (25 and 100 mg/kg) in hemiparkinsonian rats has been shown to significantly attenuate l-DOPA-induced AIMS at all time points (all $p < 0.05$) compared to l-DOPA alone. Importantly, WAY100635 reversed ±8-OH-DPAT’s effects on AIMS at all time points (all $p < 0.05$).
enhance striatal glutamate levels (Jonkers et al., 2002; Robelet et al., 2004). Here we show a similar striatal glutamate-enhancing effect (Fig. 3A and B) with a more clinically-relevant dose of l-DOPA (12 mg/kg) that is paralleled with the induction of dyskinesia (Fig. 3C). Although glutamate itself is not sufficient to induce dyskinesia when perfused within the DA-denervated striatum (Buck et al., 2010), it may serve to modulate dyskinesia behavior, as suggested by studies reporting that direct intrastratal administration of glutamate antagonists reduce LID (Marin et al., 1996; Nash & Brocthe, 2000).

Similar to l-DOPA, D1R agonists induce dyskinesia (Rascol et al., 2001, 2006; Delfino et al., 2007; Dupre et al., 2007, 2008a) and increase striatal glutamate receptor binding in parkinsonian monkeys (Calon et al., 2002); however, their effects on striatal glutamate release in the DA-depleted brain have not been directly investigated. The present study is the first to report that stimulation with the D1R agonist SKF81297, while inducing dyskinesia (Fig. 4C), does not increase extracellular striatal glutamate levels compared to vehicle treatment in DA-depleted rats (Fig. 4A and B). Thus, our results indicate enhanced extracellular striatal glutamate levels are not directly related to the expression of D1R agonist-mediated dyskinesia. It is important to note that while SKF81297’s timecourse of dyskinesia is shorter (likely attributable to its pharmacokinetic profile), it induced similar severities of axial, forelimb, and orolingual AIMS compared to l-DOPA (Fig 1A, Fig 3C, Fig 4C). Therefore, the differential effects on glutamate between D1R agonism and l-DOPA cannot be explained by differences in AIMS expression. In support of our effects, glutamate antagonism failed to reduce D1R-induced locomotor activity and striatal preprotachykinin mRNA expression in parkinsonian rat pups (Campbell et al., 2006). Taken as a whole, it is possible that stimulation of both DA receptor subtypes is necessary to augment striatal glutamate, as seen in vitro where stimulation of both D1R and D2R were required to enhance glutamate-induced firing of striatal neurons (Hu & White, 1997), where population administration of D1R and D2R agonists did not affect striatal glutamate in intact animals (Yamamoto & Davy, 1992), and in vivo with apomorphine in intact rats (Porras & Mora, 1995) and l-DOPA in DA-depleted rats (current study; Robelet et al., 2004). Future work should investigate the effects of D2R agonist treatment on extracellular glutamate levels within the DA-depleted striatum, both alone and in conjunction with D1R agonism, to validate this hypothesis.

Much research has shown 5-HT1AR agonists reduce dyskinesia and most researchers have attributed this to a raphe-mediated mechanism that involves dampening the release of DA delivered to the striatum (Dekundy et al., 2007; Carta et al., 2007; Eskow et al., 2009; Lindgren et al., 2010). However, we have previously shown that local stimulation of striatal 5-HT1ARs via microinjections attenuates LID and D1R agonist-induced dyskinesia while improving l-DOPA efficacy and motor performance in hemiparkinsonian rats (Dupre et al., 2008a; Bishop et al., 2009). In the current experiments, we report for the first time that systemic and intrastratal 5-HT1AR stimulation using ±8-OH-DPAT diminishes l-DOPA-induced striatal glutamate efflux (Figs. 3A, B, 5A) while concurrently reducing LID (Figs. 3C and 5B). The intrastratal effects lasted the entire time ±8-OH-DPAT was being infused, while the systemic effects lasted for the first 120 min following injection, likely reflecting ±8-OH-DPAT’s short half-life (Yu & Lewander, 1997). Importantly, these results were reversed with the 5-HT1AR antagonist WAY100635 (Figs. 3A–C; 5A–B), indicating a specific 5-HT1AR effect. In sham-lesioned rats, pretreatment with ±8-OH-DPAT did not alter striatal glutamate (Fig. 3D and E), indicating ±8-OH-DPAT’s effects on l-DOPA-related striatal glutamate were dependent on DA-depletion. In support of our findings, striatal 5-HT1AR binding was increased in parkinsonian primates chronically treated with l-DOPA (Huot et al., 2010). This suggests a gain of function for 5-HT1AR in the striatum of DA-depleted, l-DOPA-treated animals and may explain ±8-OH-DPAT’s effects in 6-OHDA- and sham-lesioned animals.

In contrast to l-DOPA-treated, DA-depleted rats, treatment with ±8-OH-DPAT prior to SKF81297, while diminishing dyskinesia (Fig 4C), did not reduce striatal glutamate in D1R agonist-primed, 6-OHDA-lesioned rats (Fig. 4A and B). Collectively, our results suggest two pathways whereby striatal 5-HT1AR stimulation may diminish dyskinesia. First, 5-HT1AR agonists may act pre-synaptically on glutamatergic corticostriatal neurons to reduce l-DOPA-mediated glutamate efflux. Next, while this mechanism may also relate to LID, D1R agonist-mediated dyskinesia may be modulated via a postsynaptic mechanism(s) that modifies downstream effects. Indeed, 5-HT1AR stimulation may alter D1R-mediated signaling pathways within the striatum, such as those involved in ΔFosB (Cenci et al., 1998; Cao et al., 2010), zif-268 (Carta et al., 2010), and the phosphorylation of extracellular regulated kinase (ERK) 1/2 (Gerfen et al., 2002, 2008; Westin et al., 2007), and subsequent GABA release to output structures, such as the substantia nigra pars reticulata (Weick & Walters, 1986; Radnikow & Misgeld, 1998; Yamamoto et al., 2006; Rangel-Barajas et al., 2008), that are likely involved in dyskinesia caused by D1R agonists. For instance, 5-HT1AR stimulation has been shown to reduce l-DOPA-induced overactive striatal preprodynorphin mRNA that is associated with the D1R pathway (Tomijama et al., 2005; Bishop et al., 2009). Further studies are underway to test these hypotheses surrounding striatal 5-HT1AR and D1R-mediated dyskinesia.

While 5-HT1AR agonists diminish dyskinesia, these compounds have been shown to produce adverse side effects (Irvani et al., 2006), including flat body posture indicative of 5-HT syndrome (Goodwin et al., 1987; Carey et al., 2004). We used a systemic dose of ±8-OH-DPAT (1.0 mg/kg, sc) that produced transient 5-HT syndrome in the majority of rats treated with l-DOPA; however, no 5-HT syndrome was observed in rats treated in combination with SKF81297. In previous studies using unilaterally DA-depleted rats, this dose has not reduced l-DOPA-efﬁcacy on the forepaw adjusting steps test (Bishop et al., 2009) and has actually increased stepping on this test when given alone or in combination with SKF81297 (Dupre et al., 2008a). The concentrations of ±8-OH-DPAT (7.5 mM and 15 mM) for intrastriatal infusion were determined based on intrastriatal microinjection doses (5 and 10 µg) previously used to produce anti-dyskinetic effects (Dupre et al., 2008a; Bishop et al., 2009). The lower dose of ±8-OH-DPAT variably produced 5-HT syndrome, while the higher dose consistently resulted in symptoms; however, both doses attenuated l-DOPA-induced striatal glutamate efflux and LID to comparable degrees (Fig. 5A and B). Thus, while 5-HT syndrome did not appear to be indicative of the reduction in...
It remains an important factor to consider when using 5-HT1AR agonists.

**Conclusions**

Several important conclusions can be drawn from the current set of experiments. First, L-DOPA treatment produced a moderate augmentation of glutamate in the DA-depleted striatum, whereas SKF81297 did not. These results suggest that enhancement of extracellular striatal glutamate may be important for the expression of LID and not necessarily D1R-mediated dyskinesia. Moreover, stimulation of striatal 5-HT1AR reduces local L-DOPA-induced glutamate efflux while concomitantly diminishing LID. These findings, while providing insight into the striatal workings surrounding dyskinesia in PD, reveal a novel mechanism of 5-HT1AR agonists that may be useful for the treatment of LID and PD.
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