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Modulation of L-DOPA's antiparkinsonian and dyskinetic effects by $\alpha 2$ -noradrenergic receptors within the locus coeruleus

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Abstract

Long-term L-DOPA use for Parkinson's disease (PD) is frequently complicated by the emergence of a debilitating motor side effect known as L-DOPA-induced dyskinesia (LID). Accumulating evidence has implicated the norepinephrine (NE) system in the pathogenesis of LID. Here we used the unilateral 6-hydroxydopamine rat model of PD to determine the role of the $\alpha 2$ -adrenoceptors ($\alpha 2R$) in L-DOPA's therapeutic and detrimental motor-inducing effects. First, we characterized the effects of systemic $\alpha 2R$ stimulation with clonidine, or blockade with atipamezole, on LID using the rodent abnormal involuntary movements scale, and L-DOPA's therapeutic effects using the forepaw adjusting steps test and locomotor activity chambers. The anatomical locus of action of $\alpha 2R$ in LID was investigated by directly infusing clonidine or atipamezole into the locus coeruleus prior to systemic L-DOPA administration. Results showed systemic clonidine treatment reduced LID and locomotor activity but did not interfere with L-DOPA's antiparkinsonian benefits. Conversely, systemic atipamezole pretreatment prolonged LID and locomotor activity but did not modulate L-DOPA's antiparkinsonian benefits. Intra-LC infusions of clonidine and atipamezole mirrored systemic effects where clonidine reduced, and atipamezole increased, LID. Collectively, these results demonstrate that $\alpha 2R$ play an important modulatory role in L-DOPA-mediated behaviors and should be further investigated as a potential therapeutic target.

Keywords

Locus coeruleus; L-DOPA-induced dyskinesia; norepinephrine; clonidine; atipamezole; $\alpha 2$ -adrenoceptor

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1. Introduction

Dopamine (DA) replacement with L-DOPA has remained the most widely used and effective treatment for Parkinson's disease (PD) since its introduction in the 1960's [1, 2]. However, long-term L-DOPA use is associated with the emergence of a debilitating, hyperkinetic motor side effect known as L-DOPA-induced dyskinesia (LID) [3]. While the primary cause of LID is not fully understood, it is generally accepted that LID involves excessive release of L-DOPA-derived DA and stimulation of sensitized DA receptors leading to aberrant striatal signaling pathway activity [4]. Attempts to reduce LID by pharmacologically manipulating the DA system, either by blocking DA receptors or reducing L-DOPA dosage, are frequently complicated by the return of primary PD symptoms [5–7]. An alternative to this has been the exploration of non-dopaminergic targets which interact with central motor circuits.

There is a growing body of evidence implicating the norepinephrine (NE) system in the expression of LID. NE levels are frequently reduced in the PD brain [8] and L-DOPA treatment has been shown to enhance central NE concentrations [9, 10]. Recent experimental evidence reported LID expression temporally coincides with L-DOPA-derived striatal NE efflux in L-DOPA-primed, hemiparkinsonian rats [11]. As such, a number of compounds targeting the NE system, and α 2-adrenoceptors (α 2R) specifically, have shown promise for the treatment of LID. Paradoxically, systemic treatment with either agonists or antagonists for the α 2R has been shown to reduce LID symptoms, but demonstrate equivocal effects on L-DOPA's therapeutic benefits. The classic α 2R agonist clonidine relieves LID but blocks L-DOPA's anti-parkinsonian motor effects [12, 13]. In contrast, several classic α 2R antagonists including idazoxan, yohimbine, and fipamezole reduce the severity or duration of LID in experimental and clinical populations [13–19] without interfering with L-DOPA's antiparkinsonian motor benefits [20, 21]. In fact, co-treatment with the α 2R-antagonist idazoxan actually extends L-DOPA's antiparkinsonian benefits.

The precise anatomical site of action for these effects has not yet been determined. α 2R are abundantly expressed throughout central motor nuclei including the striatum and the rest of the basal ganglia [22, 23]; however, it has been suggested that α 2R regulate L-DOPA's effects via a presynaptic mechanism since α 2R antagonists influence L-DOPA-, but not DA-agonist-, induced dyskinesias [24]. The locus coeruleus (LC), the main noradrenergic nucleus in the brain, is a promising candidate since basal firing activity of NE neurons in the locus coeruleus (LC) was positively correlated with dyskinesia severity in a rodent model of LID [25]. NE neurotransmission from the LC is regulated by a class of inhibitory somatodendritic α 2-autoreceptors [26] and stimulation or blockade of these receptors have been shown to influence monoamine efflux and neurotransmitter signaling in motor regions implicated in LID [27–30].

Progress in understanding the role of α 2R in LID has been slowed due to the limited number of receptor-specific ligands. Atipamezole is a highly potent and selective α 2R antagonist demonstrating 100 times greater affinity for the α 2R than other α 2R antagonists commonly investigated in LID like idazoxan or yohimbine [31]. In order to clarify the pharmacological and neuroanatomical underpinnings of α 2R action in LID and PD, we first evaluated the

consequence of systemic administration of atipamezole, or the classic $\alpha 2R$ agonist clonidine, on L-DOPA's dyskinetic, antiparkinsonian, and general motor-activating properties. The second goal was to determine whether these effects were mediated by $\alpha 2R$ within the LC using site-specific microinfusions. Collectively, the current work demonstrated that $\alpha 2R$ modify L-DOPA's motor actions in part due to a population of receptors located in the LC.

2. Materials and Methods

2.1 Animals

Adult male Sprague-Dawley rats (N = 43; 225–250 g upon arrival; Harlan, USA) were housed in plastic cages (22 cm high, 45 cm deep, and 23 cm wide) with free access to water and standard lab chow (Rodent Diet 5001; Lab Diet, Brentwood, MO, USA). The colony room was maintained at 22–23°C on a 12 h light/dark cycle (lights on at 0700 hrs). Animals were cared for in accordance with the guidelines of the Institutional Animal Care and Use Committee at Binghamton University and the “Guide for the Care and Use of Laboratory Animals” (Institute for Laboratory Animal Research, National Academies Press 2011). Throughout this work, all efforts were made to minimize animal suffering, reduce the number of animals used and utilize alternatives to in vivo techniques when available.

2.2 Drugs

All systemic injections were administered at a volume of 1 ml/kg. All drugs were acquired from Sigma (St. Louis, MO., USA) unless otherwise noted. Buprenorphine (Rechitt Benckiser Pharmaceuticals Inc., Richmond, VA) was suspended in saline (dH₂O containing 0.9% NaCl). L-DOPA methyl ester + benserazide (hereafter denoted as L-DOPA) and 6-hydroxydopamine (6-OHDA) were dissolved in saline with 0.1% ascorbic acid (L-DOPA vehicle). Varying doses of L-DOPA were administered throughout this study, however it should be noted that L-DOPA was always co-administered with the peripheral decarboxylase inhibitor benserazide in the same vehicle at a dose of 15 mg/kg. Desipramine and clonidine were dissolved in dH₂O for systemic treatments. Clonidine was dissolved in saline when administered intracranially. Atipamezole was dissolved in a 25% dimethyl sulfoxide, 75% dH₂O solution.

2.3 Surgery

Prior to surgery, rats were treated with desipramine HCl (25 mg/kg, ip) in an attempt to protect NE neurons and buprenorphine (0.03 mg/kg ip) as pre-emptive analgesia. Under isoflurane anesthesia (2–3% in oxygen, 1000 cc/min), rats were given a unilateral DA lesion by infusing 6-OHDA (12 ug in 4 ul) directly into the medial forebrain bundle (AP, –1.8 mm; ML, 2.0mm, DV, –8.6 mm from bregma; Paxinos & Watson, 1998) as described in detail previously (Ostock et al., 2011). For rats in experiment 1, stainless steel wound clips were used to close the surgical site (n = 11). During 6-OHDA surgery, an additional two cohorts of rats in experiment 2 (N = 32; clonidine treated: n = 12; atipamezole treated: n = 18) were also implanted with a 30 gauge microinfusion guide cannulae (Plastics One, Roanoke, VA) above the LC, ipsilateral to the 6-OHDA lesion (AP, –9.8 mm; ML, +1.36, DV, –6.42 from bregma). Cannulae were fixed in place using dental acrylic (Lang Dental, Wheeling, IL) and

4 screws. Dummy cannulae (Plastics One) cut 1 mm shorter than the guide cannulae were inserted in the guide cannulae to preserve patency. Rats in experiment 1 were pair-housed while cannulated rats in experiment 2 were single-housed.

2.4 Behavioral Tests

2.4.1 Abnormal involuntary movements and rotations—The abnormal involuntary movements (AIMs) test is a well-established metric of rodent dyskinesia [14, 32]. Rats were monitored for AIMs and rotations by trained observers (95% agreement between experimenters) using a procedure modified from Cenci & Lundblad (2007) and described in detail in Lindenbach et al., (2011). During each rating period, individual dyskinesia severity scores ranging from 0 (not present) to 4 (severe and not interruptible) were given for axial, forelimb, and orolingual (ALO) dyskinesia subtypes. The sum of these subtypes was then reported as the ALO AIMs score for each time-point. Higher ALO AIMs scores are indicative of greater LID severity. During AIMs ratings, contralateral rotations, defined as complete 360° turns away from the lesioned side of the brain were recorded.

2.4.2 Forepaw adjusting steps—Rodent akinesia manifests as deficient stepping ability on the side of the body contralateral to brain lesion in the forepaw adjusting steps (FAS) test [33]. L-DOPA treatment reverses stepping deficits; therefore, FAS can be used to determine whether an adjunctive therapy is interfering with L-DOPA's antiparkinsonian effects. Using a procedure similar to that described previously [34], the experimenter held the rat's rear torso and one forelimb while the free forelimb was forced to bear the rat's body weight. Rats were moved laterally across a table at a steady rate of 90 cm/10 s and the number of adjusting steps taken by each forelimb to compensate for lateral movement was counted by a trainer observer. Each session consisted of six trials per forelimb alternating between directions where forehand steps were defined as weight-bearing steps made towards the body and backhand steps were defined as steps taken away from the body. Stepping data were expressed as % intact stepping (lesioned steps/ intact steps). Lower percent intact scores reflect greater forelimb akinesia.

2.4.3 Locomotor Chambers—Drug-induced changes in spontaneous and L-DOPA-induced locomotor behaviors were assessed using activity chambers (Accuscan Instruments, Columbus, OH) as described previously (Lindenbach et al., 2011). Six Acrylic chambers (41 × 41 × 30.5 cm) surrounded by infrared photocell arrays synched with Versamax and Versadat software were used to measure locomotor activity. Photo beam breaks were recorded to assess horizontal and vertical patterns of movement.

2.5 Tissue collection and processing

One week after behavioral analyses were completed, all rats were killed by rapid decapitation and brains were immediately removed. Left and right striatal tissue was freshly dissected and stored at -80°C for analysis of monoamine content using high-performance liquid chromatography (HPLC-ED) according to the protocol of Bishop et al. [35] (based on Kilpatrick et al. [36]). Samples were homogenized in ice cold perchloric acid (0.1 M) with 1% ethanol and 0.02% EDTA and spun for 45 min at 14,000 g at 4°C. Aliquots of supernatant were then analyzed for abundance of NE, DA, and the DA metabolite 3,4-

dihydroxyphenylacetic acid (DOPAC) using HPLC-ED. The final oxidation current values were compared to known standards ($1\text{e}6 - 1\text{e}9$) and adjusted according to striatal tissue weights.

Verification of proper cannulae placement for rats in experiment 2 was accomplished using cresyl violet staining. Posterior portions of the brain were removed and rapidly frozen in methylbutane (-30°C) then stored at -20°C for evaluation of LC cannulae placements. Cresyl violet (FD, Neurotechnologies, Baltimore, MD) staining was used to determine injection sites from cryostat-generated $20\text{-}\mu\text{m}$ coronal sections post-fixed with 4% paraformaldehyde (Fisher Scientific, Hanover Park, IL).

2.6 General Experimental Procedures

A flowchart of the experimental design is shown in Figure 1.

2.6.1 Experiment 1: Effect of systemic $\alpha 2\text{R}$ stimulation or blockade on L-DOPA-mediated behaviors—The effects of the $\alpha 2\text{R}$ agonist clonidine and the $\alpha 2\text{R}$ antagonist atipamezole on L-DOPA's dyskinetic and therapeutic effects were evaluated using a counterbalanced, within-subjects design by investigators blind to treatment condition. Three weeks after surgery, rats were treated with L-DOPA (12 mg/kg , sc) for 7 days to rapidly induce stable LID [37]. Starting 4 days later and continuing until all rats received all treatment combinations, the effects of clonidine or atipamezole on L-DOPA mediated behaviors was first assessed for LID and then for akinesia and motor activity. On AIMs test days (days 32–65), rats were treated with clonidine ($0, 0.01, 0.1\text{ mg/kg}$, ip) or atipamezole ($0, 0.3, 1.0\text{ mg/kg}$, sc) followed 30 min later by L-DOPA ($4, 6\text{ mg/kg}$, sc) and were rated for ALO AIMs and rotations every 10 min for 3 h post L-DOPA treatment. The effects of clonidine and atipamezole on akinesia and motor performance were assessed using the FAS test and locomotor activity chambers, respectively. Rats were habituated to the FAS testing parameters and the locomotor chambers off treatment during the 2 weeks preceding this testing. On test days, rats were injected with either clonidine ($0, 0.1\text{ mg/kg}$, ip) or atipamezole ($0, 1.0\text{ mg/kg}$, sc) followed by L-DOPA ($0, 4, 6\text{ mg/kg}$, sc) 30 min later. On FAS test days, the effects of clonidine or atipamezole were evaluated 60 min post L-DOPA treatment. For tests of motor activity, rats were placed into the activity chambers immediately following L-DOPA injection. Two animals died prior to completing all FAS and activity chamber test days and their data was excluded from those analyses.

2.6.2 Experiment 2: Effect of Intra-LC $\alpha 2\text{R}$ stimulation and blockade on LID—Two separate cohorts of rats were implanted with cannulae over the left LC in addition to the unilateral 6-OHDA MFB lesion. Only rats with correct cannula placement, as determined by cresyl violet histology at the the experiment, were retained for final data analyses (clonidine rats: $n = 8$; atipamezole treated rats, $n = 11$). Three weeks after surgery all rats were primed with L-DOPA (12 mg/kg) for 1 week to rapidly induce stable ALO AIMs. Rats were tested with the dose of L-DOPA used throughout test days 2 days later (clonidine treated rats: L-DOPA 6 mg/kg , sc; atipamezole treated rats: L-DOPA 4 mg/kg , sc) and rated for AIMs. L-DOPA doses were chosen for clonidine and atipamezole microinfusion tests by selecting the doses of L-DOPA that were modified by systemic $\alpha 2\text{R}$ -

treatment and produced a similar magnitude of effect in experiment 1. The effects of direct LC infusions of clonidine or atipamezole on dyskinesia were tested in two separate cohorts of rats. Treatments were counterbalanced across days for each cohort using a Latin-square design for the doses described in Table 1. Intra-LC clonidine microinfusion tests occurred on 4 separate test days where clonidine or its vehicle was administered into the LC prior to systemic L-DOPA (6 mg/kg) treatment (C0-LD6, C2-LD6, C20-LD6) or the high dose of clonidine was administered into the LC prior to systemic L-DOPA vehicle treatment (C20-LD0). Since a floor effect was expected for clonidine treatment based on previous behavioral data, intra-LC clonidine followed by systemic L-DOPA vehicle (C20-LD0) treatment was used as the control group. Intra-LC atipamezole tests occurred on 5 separate test days as follows: atipamezole or its vehicle was injected into the LC 5 min prior to systemic L-DOPA (4 mg/kg) (A0-LD4, A1.5-LD4, A15.0-LD4) or L-DOPA vehicle (A0-LD0, A15.0-LD0) treatment. Given the possibility that atipamezole itself might induce dyskinesia, a comparison between intra-LC atipamezole without L-DOPA (A0-LD0) and vehicle-vehicle (A0-LD0) was also examined.

During LC microinfusions, rats were restrained with a towel and an injector was lowered 1 mm past the end of the guide cannula. Drugs were infused at a rate of 0.4 μ l/min for a total volume of 1.0 μ l in 2.5 min using a microinfusion pump (Harvard Apparatus, Boston, MA) holding one 10 μ l Hamilton syringe attached to plastic tubing (PE20 Tygon tubing; Plastics One) and the injector. The injector was removed 5 min after the flow was stopped. Rats were then immediately injected with L-DOPA (clonidine treated rats: 0, 6 mg/kg; atipamezole treated rats: 0, 4 mg/kg, sc) and were rated for AIMs and rotations every 10 min for 3 h.

2.7 Statistical Analyses

Non-parametric ALO AIMs data were evaluated using within-subjects Friedman tests at each time point, and overall effects for experiments 1 and 2. Wilcoxon sign-rank post hoc were used to distinguish significant differences. Parametric rotation, FAS, and locomotor chamber data were analyzed using repeated-measures, within-subjects ANOVAs. Fisher's LSD post hoc comparisons were used to evaluate significant differences. Striatal monoamine and metabolite concentrations were analyzed using dependent samples t-tests. Analyses were performed with the use of Statistica software '98 (Statsoft Inc., Tulsa, OK, USA) and alpha was set at 0.05.

3. Results

3.1 Monoamine and metabolite levels

The effects of 6-OHDA lesion on concentrations of NE, DA, and DOPAC levels in the lesioned (left) versus intact (right) striatum are reported in Table 2.

As expected, unilateral 6-OHDA injection into the MFB produced significant (~99%) reductions in DA and the DA metabolite DOPAC in the lesioned striatal hemisphere of all rats compared to the intact striatal hemisphere ($t_{25} = -9.51; 9.15, p < 0.001$). Despite

desipramine pretreatment ~80% reductions in striatal NE levels were also observed ipsilateral to MFB lesion ($t_{25} = 4.33$, $p < 0.001$).

3.2 Experiment 1: Effect of systemic α_2R stimulation or blockade on L-DOPA-mediated behavior

3.2.1 ALO AIMs and rotations—Upon examination of the effects of the α_2R agonist clonidine, time-point analysis revealed significant differences in 4 mg/kg L-DOPA-induced ALO AIMs expression at the 20–100, 120, and 170 min time-points, as well as for the total ALO AIMs sum ($\chi^2 > 6.46$; $p < 0.05$ for all). As shown in Figure 2A, pretreatment with the high dose of clonidine (0.1 mg/kg; C0.1-LD) significantly reduced ALO AIMs expression compared to the low dose (0.01 mg/kg; C0.01-LD) or vehicle (0 mg/kg; C0-LD) throughout the first 100 min of testing. Additionally, the high dose of clonidine also reduced ALO AIMs at 120 and 170 min time-points versus vehicle. The antidyskinetic effects of clonidine on the 6 mg/kg dose of L-DOPA were less pronounced. As shown in Figure 2C, significant differences in ALO AIMs expression were found at the 30 and 40 min time-points as well as the total ALO AIMs sum ($\chi^2 = 10.12, 10.32, 10.82$, respectively; $p < 0.01$ for all). Post hoc analyses revealed pretreatment with both doses of clonidine (0.01, 0.1 mg/kg) significantly reduced ALO AIMs expression at both the 30 min time-point, and overall, when compared to vehicle. Additionally, the high dose of clonidine also reduced ALO AIMs expression at the 40 min time-point compared to vehicle pretreatment ($p < 0.05$ for all).

For rotations, the 3(treatment) $\times$ 18(time) within-subjects repeated-measures ANOVAs revealed significant main effects of clonidine treatment ($F_{2, 16} = 4.52$, $p < 0.05$; $F_{2, 18} = 8.67$, $p < 0.01$), time ($F_{17, 136} = 6.49$, $p < 0.001$; $F_{17, 153} = 8.35$, $p < 0.001$) and significant treatment by time interactions ($F_{34, 272} = 3.13$, $p < 0.01$; $F_{34, 306} = 1.53$, $p < 0.05$) for L-DOPA 4 and 6 mg/kg, respectively. Clonidine pretreatment (0.01, 0.1 mg/kg) reduced L-DOPA (4 mg/kg)-induced rotations compared to vehicle at the 40 and 50 min time-points (Figure 2B). The high dose of clonidine also attenuated rotational behavior from 60–100 min compared to both the low dose and vehicle. Clonidine produced similar effects on rotations induced by the 6 mg/kg dose of L-DOPA. As shown in Figure 2D (insert), clonidine pretreatment dose dependently attenuated total rotations. Post-hoc analysis of the time-course revealed pretreatment with the high dose of clonidine attenuated rotations from 60–130 min, while the low dose of clonidine decreased rotations at the 60–90, and 120 min time-points versus vehicle.

When atipamezole was administered prior to 4 mg/kg L-DOPA, analyses revealed significant differences in ALO AIMs expression at the 30, 40, 60, 120, and 140 min time-points, as well as the total ALO AIMs sum ($\chi^2 > 6.45$ for all; all $p < 0.05$, Figure 2E). Post hoc tests indicated the low dose of atipamezole (0.3 mg/kg; A0.3-LD) enhanced ALO AIMs expression at 60 and 120 min post L-DOPA, compared to vehicle (0 mg/kg; A0-LD) pretreatment. Pretreatment with the high dose of atipamezole (1.0 mg/kg; A1.0-LD) enhanced ALO AIMs expression at 30, 40, 120, and 140 min post L-DOPA, compared to vehicle ($p < 0.05$ for all). In addition, evaluation of the total ALO AIMs summed over the 3 h period revealed that pretreatment with both doses of atipamezole significantly enhanced ALO AIMs expression compared to vehicle (Figure 2E, insert). In Figure 2G, only 1 time-

point showed a statistically significant distinction when atipamezole was co-administered with 6 mg/kg L-DOPA (130 min: $\chi^2 = 6.86$, $p < 0.05$), with no significant effects on the total sum or time-course of ALO AIMs. Post hoc analysis at the 130 min time-point revealed that ALO AIMs were higher when pretreated with vehicle, than the low (0.3 mg/kg) dose of atipamezole ($p < 0.05$).

Analysis of the effects of atipamezole on L-DOPA-induced rotations using $3(\text{treatment}) \times 18(\text{time})$ within-subjects repeated-measures ANOVA revealed significant main effects of treatment ($F_{2, 18} = 4.17$, $p < 0.01$) and time ($F_{17, 153} = 6.15$, $p < 0.01$), but not an interaction, for L-DOPA 4 mg/kg (Figure 2F). Post hoc analyses determined co-treatment of 4 mg/kg L-DOPA with the 0.1 mg/kg dose of atipamezole significantly enhanced contralateral rotations, compared to vehicle ($p < 0.05$; Figure 2F, insert). A significant effect of time ($F_{17, 153} = 5.96$, $p < 0.01$), but not treatment, and no interaction was observed with 6 mg/kg L-DOPA (Figure 2H).

3.2.2 FAS—The FAS test was used to determine whether systemic treatment with clonidine or atipamezole altered L-DOPA's restorative motor benefits. Percent-intact forehand-stepping was analyzed using one-factor, repeated-measures ANOVAs and demonstrated main effects of treatment for both clonidine ($F_{5, 45} = 6.79$, $p < 0.001$) and atipamezole ($F_{5, 45} = 9.72$, $p < 0.001$). A limited number of post-hoc comparisons were examined further. As shown in Figures 3A and 3B, forehand stepping deficits were dose-dependently reversed by both the low (C0-LD4, A0-LD4) and high (C0-LD6, A0-LD6) doses of L-DOPA compared to vehicle (C0-LD0, A0-LD0).

Treatment with clonidine or atipamezole alone (C0.1-LD0; A1.0-LD0) did not alter forehand stepping compared to L-DOPA vehicle treatment alone (A0-LD0; C0-LD0). Moreover, when combined with either dose of L-DOPA (4, 6 mg/kg) neither clonidine nor atipamezole altered L-DOPA's motor restorative effects on forehand stepping. For percent backhand stepping, a significant effect of treatment was found for clonidine ($F_{5, 45} = 8.32$, $p < 0.001$), but not atipamezole (Figure 3D). As shown in Figure 3C, the high dose of L-DOPA enhanced backhand stepping compared to vehicle. In addition, co-administration of clonidine with L-DOPA 4 mg/kg (C0.1-LD4) improved backhand stepping beyond the motor benefits achieved with L-DOPA 4 mg/kg treatment alone (C0-LD4).

3.2.3 Locomotor activity—The effects of clonidine and atipamezole pretreatment on motor behaviors were assessed in locomotor activity chambers (Figure 4). Data were collapsed into 3 1 h time blocks for each metric and analyzed using $6(\text{treatment}) \times 3(\text{time})$ within-subjects repeated-measures ANOVAs. A limited number of post hoc comparisons were conducted to further evaluate significant effects.

For clonidine treatment, analysis of distance traveled demonstrated a significant effect of treatment ($F_{5, 35} = 8.87$, $p < 0.001$) and a significant treatment by time interaction ($F_{10, 70} = 2.83$, $p < 0.01$). As shown in Figure 4A, regardless of dose, L-DOPA alone (C0-LD4, C0-LD6) enhanced distance traveled compared to vehicle treatment (C0-LD0) in the first 2 h. C0-LD6 also increased distance traveled during the third hour compared to C0-LD0. Administration of clonidine with the low dose of L-DOPA (C0.1-LD4) attenuated

movement to control (C0.1-LD0) levels for the entirety of the test. A similar reduction in movement was seen during the first hour when clonidine was administered with the higher dose of L-DOPA (C0.1-LD6; $p < 0.05$ for all). Significant main effects of treatment ($F_{5, 35} = 4.66$, $p < 0.01$), time ($F_{2, 14} = 7.35$, $p < 0.01$), and a treatment by time interaction ($F_{10, 70} = 3.60$, $p < 0.001$) were also observed for vertical activity (Figure 4B). Closer inspection revealed that significantly more vertical activity occurred following the low dose of L-DOPA (C0-LD4), than C0-LD0 or the high dose of L-DOPA (C0-LD6), during the first and second hours. Surprisingly, C0-LD6 treatment did not significantly augment vertical activity compared to C0-LD0 treatment in either the first or second hour. However, clonidine pretreatment reduced vertical activity during the first hour when given alone (C0.1-LD0) versus C0-LD0, and in conjunction with either dose of L-DOPA (C0.1-LD4, C0.1-LD6) when compared to C0-LD4, and C0-LD6, respectively. In the second hour, the C0.1-LD4-induced suppression of vertical activity was still observed compared to V-LD4 ($p < 0.05$ for all). Analysis of stereotypy count revealed significant effects of treatment ($F_{5, 35} = 15.63$, $p < 0.001$), time ($F_{2, 14} = 6.24$, $p < 0.05$), and a treatment by time interaction ($F_{10, 70} = 4.47$, $p < 0.001$). As shown in Figure 4C, both C0-LD4 and C0-LD6 enhanced stereotypic behaviors during the first 2 h, compared to C0-LD0. L-DOPA (4, 6 mg/kg)-induced stereotypy was reduced in the first 2 h when clonidine 0.1 mg/kg (C0.1-LD4, C0.1-LD6) was administered. This effect was most pronounced in the first hour, where C0.1-LD4 and C0.1-LD6 treatment did not differ from C0-LD0. In the third hour, stereotypic behaviors were still elevated in rats that received C0-LD6. As in the first 2 h, C0.1-LD6 treatment significantly reduced stereotypic behaviors compared to C0-LD6 ($p < 0.05$ for all).

For atipamezole, analysis of distance traveled (Figure 4D) revealed a significant effect of treatment ($F_{5, 35} = 7.73$, $p < 0.001$), time ($F_{2, 14} = 4.45$, $p < 0.05$), and a treatment by time interaction ($F_{10, 70} = 2.08$, $p < 0.05$). Compared to vehicle treatment (A0-LD0), all treatments with L-DOPA (A0-LD4, A0-LD6, A1.0-LD4, A1.0-LD6) enhanced the distance traveled in the first two hours. However, distance traveled in the third hour of testing was no longer elevated in rats that received L-DOPA 4 mg/kg (V-LD4, A1.0-LD4) compared to A0-LD0. Interestingly, during the second hour, A1.0-LD6 treatment further enhanced distance traveled compared to L-DOPA alone (A0-LD6; $p < 0.05$ for all). Analysis of vertical activity (Figure 4E) revealed a significant main effect of time ($F_{2, 14} = 6.31$, $p < 0.05$), but no effect of treatment and no interaction. Significant main effects of treatment ($F_{5, 35} = 4.33$, $p < 0.001$), time ($F_{2, 14} = 27.11$, $p < 0.05$), and a treatment by time interaction ($F_{10, 70} = 10.81$, $p < 0.001$) were found for stereotypy count (Figure 4F). Analysis of the interaction revealed that regardless of pretreatment, L-DOPA induced significantly more stereotypic behaviors in the first 2 h compared to vehicle (A0-LD0). In the 3rd h, stereotypic behaviors remained elevated only in rats that received the high dose of L-DOPA (A0-LD6, A1.0-LD6) or the low dose of L-DOPA with atipamezole (A1.0-LD4) ($p < 0.05$ for all).

3.3 Experiment 2: Effect of intra-LC $\alpha 2R$ stimulation or blockade on L-DOPA-mediated behavior

3.3.1 ALO AIMs and rotations—Friedman tests at each individual time-point were used to evaluate the time-course of effects of intra-LC clonidine and atipamezole infusion on L-DOPA-induced ALO AIMs (for representative cannulae placements see Figure 5A). Direct

infusions of clonidine into the LC reduced dyskinesia during the first 50 min and overall ($\chi^2 > 6.00$, all $p < 0.05$; Figure 5B). The effects of intra-LC clonidine were dose-dependent. The high dose of clonidine (20 μg) reduced ALO AIMs at the 10, 20, 40, and 50 min time-points as well as the total ALO AIMs sum (inset) compared to vehicle. The low dose reduced ALO AIMs at the 50th min as well as the total overall ALO AIMs sum. While there was a significant effect of time on rotations ($F_{17, 119} = 3.15$; $p < 0.001$), there was not an effect of treatment, nor a time by treatment interaction (Figure 5D).

For atipamezole treated rats, significant treatment effects were observed for the first 150 min and the total sum ($\chi^2 > 11.43$; $p < 0.05$ for all). A limited number of Wilcoxon sign-rank post-hoc tests were performed including comparisons of: intra-LC atipamezole (1.5, 15 μg) plus systemic L-DOPA (4 mg/kg; A1.5-LD, A15.0-LD) versus intra-LC vehicle plus systemic L-DOPA (4 mg/kg; A0-LD). Since systemic atipamezole alone had activational effects on motor behaviors, the effects of atipamezole by itself (intra-LC atipamezole (15 μg) plus systemic L-DOPA vehicle (A15.0-LD0) treatment) on ALO AIMs were compared to the control, intra-LC vehicle plus systemic vehicle (A0-LD0). As depicted in Figure 5C, the high dose of atipamezole (15 μg) significantly enhanced L-DOPA-induced ALO AIMs expression at the 30 and 50 min time-points as well as overall compared to A0-LD4 treatment. The low dose of atipamezole (1.5 μg) increased L-DOPA-induced ALO AIMs at the 40 and 120 min, and showed a non-significant trend towards increased total ALO AIMs compared to A0-LD4 ($p = 0.055$; Figure 5C, insert). Interestingly, intra-LC atipamezole alone actually induced mild dyskinesia during the first 20 min compared to A0-LD0 treatment. Analysis of contralateral rotations using a 5(treatment) $\times$ 18(time) within-subjects repeated-measures ANOVA demonstrated no significant main effect of treatment or time, and no interaction (Figure 5E).

4. Discussion

Although $\alpha_2\text{R}$ have received attention in recent years as a target for modulating L-DOPA's effects in the PD brain, their mechanism(s) of action have remained elusive. In the present work, employing the selective $\alpha_2\text{R}$ antagonist atipamezole allowed us to differentiate the specific contribution of $\alpha_2\text{R}$ in L-DOPA's dyskinesia- and motor-inducing effects. Systemic $\alpha_2\text{R}$ blockade with atipamezole extended the dyskinetic and pro-locomotor actions of a low dose of L-DOPA. Conversely, $\alpha_2\text{R}$ stimulation with clonidine reduced L-DOPA-mediated locomotor and dyskinetic behaviors. For the first time, we demonstrated these effects are partially mediated by $\alpha_2\text{R}$ within the LC since direct infusions of clonidine or atipamezole reduced, and enhanced LID expression, respectively.

Several noradrenergic compounds have shown promise for the treatment of LID [13–17, 38–40]. Some $\alpha_2\text{R}$ antagonists have even made it as far as Phase II clinical trials with limited success [19, 41, 42]. Paradoxically, both agonists and antagonists for the $\alpha_2\text{R}$ display antidyskinetic actions in preclinical settings. Therefore, the first goal of the present work was to characterize the behavioral effects of two compounds with opposing actions on the $\alpha_2\text{R}$. The effects of atipamezole on LID had not previously been evaluated, however an extensive body of literature previously demonstrated $\alpha_2\text{R}$ antagonists reduce LID symptoms [13–15, 39, 42]. In the current investigation, we found atipamezole mildly enhanced LID

severity and L-DOPA-induced activity. Systemic atipamezole when combined with low dose L-DOPA actually induced an earlier onset and slower decline of LID symptoms compared to L-DOPA alone. In addition, L-DOPA-induced stereotypic behaviors, indicative of LID, were enhanced by atipamezole treatment in the locomotor chambers. This is similar to what was shown in a primate model of LID where the $\alpha 2R$ antagonist fipamezole exacerbated non-disabling dyskinesia associated with prolonged L-DOPA “on-time” [20]. In rats, others have demonstrated $\alpha 2R$ blockade using idazoxan reduced LID severity and L-DOPA-induced rotations during the first hour of testing, but actually extended the duration of LID symptoms during the last hour of testing [16]. It should be noted that many of the $\alpha 2R$ compounds previously used to investigate LID also display agonist actions at 5-HT 1_A receptors (5-HT 1_A R) and/or antagonistic properties at DA D2 receptors (DA D2R) [31, 43–46], both of which are highly implicated in LID [34, 35, 47–51]. In contrast, atipamezole is a highly potent and selective $\alpha 2R$ antagonist with virtually no affinity for DA D2R or 5-HT 1_A R [31, 45].

Previous experimental evidence demonstrates $\alpha 2R$ stimulation with clonidine suppresses LID symptoms [12, 13]. In the current investigation, we confirmed that systemic clonidine reduced L-DOPA-induced ALO AIMs and stereotypic behaviors. This effect was especially pronounced when clonidine was administered with the low dose of L-DOPA. While often reported to be a specific $\alpha 2R$ agonist, clonidine has also been shown to have actions at imidazoline receptors, $\alpha 1$ noradrenergic receptors, and 5-HT 1_A R [45, 52, 53]. In the rat, clonidine is 56 times more selective for $\alpha 2R$ than 5-HT 1_A R, where it displays weak agonist properties [45]. While not evaluated in the current investigation, high doses of clonidine could be influencing LID through interactions with the 5-HT system, which could be tested in future studies by simultaneously blocking the 5-HT 1_A R.

Coincident with the dyskinesia investigation, we employed multiple behavioral assays to examine whether $\alpha 2R$ -targeting treatments interfered with L-DOPA’s motor-restoring properties, since such actions would limit the usefulness of these compounds as L-DOPA adjuncts. One key feature of $\alpha 2R$ antagonists which has led to continued scientific interest is that they generally do not interfere with L-DOPA’s motor benefits. In fact, $\alpha 2R$ blockade with idazoxan or fipamezole has been shown to extend the length of L-DOPA’s antiparkinsonian benefits in response to a single dose of L-DOPA [17, 20, 21, 24]. In the present work, systemic atipamezole treatment did not change L-DOPA’s antiparkinsonian stepping effects on the FAS test. Atipamezole potentiated L-DOPA’s motor-enhancing effects for distance traveled in the locomotor chambers and for contralateral rotations observed during the AIMs test when given in conjunction with the low dose of L-DOPA. These activational effects support past work showing that $\alpha 2R$ blockade augments L-DOPA’s rotational behavior in hemiparkinsonian rats [54]. In contrast, clonidine pretreatment reduced L-DOPA’s motor-stimulating effects on rotations during the AIMs test, and distance traveled and vertical activity in the locomotor chambers, two metrics of general motor activity. This is in agreement with previous studies demonstrating the antiparkinsonian and rotational behaviors induced by L-DOPA or other dopaminergic drugs is reduced by concomitant $\alpha 2R$ stimulation [54–58]. Interestingly, systemic clonidine pretreatment did not hinder the low dose of L-DOPA’s antiparkinsonian effects on the FAS test at a time when dyskinesia symptoms were still suppressed.

Inhibitory $\alpha 2R$ are abundantly expressed throughout the brain, especially in regions implicated in PD like the basal ganglia and LC [22, 59–61], however the role of $\alpha 2R$ within motor circuits in modulating L-DOPA's actions are not fully understood and may well be site-specific. Here, we demonstrate a discrete population of $\alpha 2R$ within the LC influence LID. $\alpha 2R$ are abundantly expressed on NE neurons of the LC where they act as autoreceptors to control the release of NE [23, 26, 59]. We administered clonidine and atipamezole directly into the LC prior to systemic L-DOPA treatment. While in the same direction as the systemic effects, the magnitude of the intra-LC clonidine and atipamezole effects on LID were less pronounced. There was significant (~80%) loss of striatal NE levels ipsilateral to 6-OHDA infusion in the current investigation which may have led to the dampened responses. However, intra-LC clonidine did reduce L-DOPA-induced ALO AIMs expression during the first 50 min, with the greatest suppression seen in the first half-hour. In contrast, intra-LC atipamezole infusion induced an earlier onset of LID. Interestingly, intra-LC infusion of atipamezole by itself induced mild, but significant ALO AIMs expression during the first 20 min of AIMs testing compared to rats that received intra-LC vehicle followed by systemic vehicle. This supports a presynaptic mechanism through which $\alpha 2R$ can mediate the behavioral effects of L-DOPA. However, different populations of $\alpha 2R$ may play opposing roles in L-DOPA's motor effects. $\alpha 2R$ are also found in great number on GABAergic medium spiny projecting neurons in the striatum [62–64] and direct infusion of the $\alpha 2R$ antagonist idazoxan into the striatum reduced LID in a rodent model of LID [11]. While not directly tested in the current work, it is important to determine whether the antidyskinetic and antiparkinsonian effects of $\alpha 2R$ -targeting compounds can be dissociated to improve patient outcomes.

The mechanisms through which $\alpha 2R$ influence L-DOPA's behavioral effects have not yet been determined; however, NE transmission is thought to be involved. Indeed, LID has been shown to temporally coincide with L-DOPA-derived striatal NE efflux [11] and direct infusion of NE alone into the striatum has been shown to induce dyskinesia in L-DOPA-primed, hemiparkinsonian rats [11, 65]. This is further supported by work showing basal firing activity in the LC positively correlates with LID severity in a rodent model of LID [25]. At the level of the LC, $\alpha 2R$ function as autoreceptors to control the release of NE. Stimulation of $\alpha 2R$ reduces while blockade enhances central NE release [66–68]. The current body of work supports a potential NE-dependent mechanism by which $\alpha 2R$ modulate L-DOPA-mediated behaviors.

There is evidence $\alpha 2R$ in the LC influence striatal DA signaling, however it is not clear how this is occurring. $\alpha 2R$ blockade with atipamezole has been shown to potentiate L-DOPA's effects on evoked DA release in the striatum, and may enhance DA transmission via increasing DA accumulation and the size of the readily releasable DA storage pool [28]. Moreover, local and systemically administered $\alpha 2R$ antagonists have been shown to enhance striatal DA content and overflow in intact and DA-lesioned rats [29, 69, 70] while $\alpha 2R$ agonism reduced striatal DA release in experimental models [27, 71]. In contrast to this, a recent experiment using microdialysis has shown striatal infusion of the $\alpha 2R$ antagonist idazoxan reduced LID and striatal peak L-DOPA-induced striatal DA efflux [11]. LC NE neurons terminating in the striatum may influence striatal DA levels by modifying DA uptake. Indeed, evidence shows that striatal NE transporters (NET) can take up L-

DOPA-derived DA in the DA-depleted brain [72] and subsequent NET blockade exacerbated LID expression in a rodent model of LID [73]. NE from the LC could also modulate striatal DA signaling indirectly by regulating striatal DA release from the SNc [74, 75]. However, in the 6-OHDA rat model used in the current investigation, nigrostriatal DA projections are greatly depleted and L-DOPA-derived DA release is thought to be mediated by non-dopaminergic neurons. In fact, 5-HT neurons of the dorsal raphe nucleus (DRN) take up, convert, and release exogenously administered L-DOPA-derived DA into the striatum [76–78]. The LC and DRN are linked by reciprocal projections. $\alpha 1$ noradrenergic receptors are abundantly expressed on 5-HT cell bodies while $\alpha 2R$ found presynaptically on NE neurons synapsing in the dorsal raphe nucleus (DRN) [22, 79, 80]. As such, the firing activity of 5-HT neurons in the DRN is mediated by tonic activation from noradrenergic neurons that mediated by $\alpha 1$ - and $\alpha 2R$ [81, 82]. Given the DRN is a major source of L-DOPA-derived DA, it is plausible the LC may modulate LID indirectly by altering 5-HT neurotransmission in the PD brain. Direct confirmation of the impact of LC $\alpha 2R$ stimulation or blockade on striatal DA or NE efflux, and whether these effects occur trans-synaptically through the DRN are clearly warranted.

It has become increasingly apparent that L-DOPA's effects are conveyed via complex interactions between many neurotransmitter systems. In the present investigation we evaluated the role of $\alpha 2R$ in L-DOPA-mediated behaviors, and showed $\alpha 2R$ stimulation reduced, while blockade enhanced, LID symptoms and motor activity. These effects were at least partially mediated by a population of $\alpha 2R$ located within the LC. How this translates into clinical use remains an open question. To date, two $\alpha 2R$ antagonists, fipamezole and idazoxan, have proceeded to clinical trials without complete understanding of the mechanism(s) through which they are acting. These have shown only mild, if any, antidyskinetic efficacy [19, 41, 42]. Thus, more work determining how and where $\alpha 2R$ alter motor circuits in the dyskinetic brain should provide valuable information to optimize long-term use of L-DOPA.

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References

1. Katzenschlager R, Lees AJ. Treatment of Parkinson's disease: levodopa as the first choice. *Journal of neurology*. 2002; (249 Suppl 2):II19–II24. [PubMed: 12375059]
2. Hauser RA. Levodopa: past, present, and future. *European neurology*. 2009; 62:1–8. [PubMed: 19407449]
3. Jankovic J. Motor fluctuations and dyskinesias in Parkinson's disease: clinical manifestations. *Movement disorders : official journal of the Movement Disorder Society*. 2005; (20 Suppl 11):S11–S16. [PubMed: 15822109]
4. Huot P, Johnston TH, Koprich JB, Fox SH, Brotchie JM. The pharmacology of L-DOPA-induced dyskinesia in Parkinson's disease. *Pharmacological reviews*. 2013; 65:171–222. [PubMed: 23319549]
5. Grondin R, Doan VD, Gregoire L, Bedard PJ. D1 receptor blockade improves L-dopa-induced dyskinesia but worsens parkinsonism in MPTP monkeys. *Neurology*. 1999; 52:771–776. [PubMed: 10078726]

6. Elliott PJ, Walsh DM, Close SP. Dopamine D1 and D2 receptor interactions in the MPTP-treated marmoset. *Neuroscience letters*. 1992; 142:1–4. [PubMed: 1357609]
7. Goetz CG, Tanner CM, Klawans HL. Drug holiday in the management of Parkinson disease. *Clin Neuropharmacol*. 1982; 5:351–364. [PubMed: 6760963]
8. Zarow C, Lyness SA, Mortimer JA, Chui HC. Neuronal Loss Is Greater in the Locus Coeruleus Than Nucleus Basalis and Substantia Nigra in Alzheimer and Parkinson Diseases. *Arch Neurol*. 2003; 60:5.
9. Chalmers JP, Baldessarini RJ, Wurtman RJ. Effects of L-dopa on norepinephrine metabolism in the brain. *Proceedings of the National Academy of Sciences of the United States of America*. 1971; 68:662–666. [PubMed: 5276777]
10. Bianco LE, Wiesinger J, Earley CJ, Jones BC, Beard JL. Iron deficiency alters dopamine uptake and response to L-DOPA injection in Sprague-Dawley rats. *Journal of neurochemistry*. 2008; 106:205–215. [PubMed: 18363828]
11. Wang Y, Wang HS, Wang T, Huang C, Liu J. L-DOPA-induced dyskinesia in a rat model of Parkinson's disease is associated with the fluctuational release of norepinephrine in the sensorimotor striatum. *Journal of neuroscience research*. 2014
12. Gomez-Mancilla B, Bedard PJ. Effect of nondopaminergic drugs on L-DOPA-induced dyskinesia in MPTP-treated monkeys. *Clin Neuropharmacol*. 1993; 16:418–427. [PubMed: 8106150]
13. Dekundy A, Lundblad M, Danysz W, Cenci MA. Modulation of L-DOPA-induced abnormal involuntary movements by clinically tested compounds: further validation of the rat dyskinesia model. *Behavioural brain research*. 2007; 179:76–89. [PubMed: 17306893]
14. Lundblad M, Andersson M, Winkler C, Kirik D, Wierup N, Cenci MA. Pharmacological validation of behavioral measures of akinesia and dyskinesia in a rat model of Parkinson's disease. *The European journal of neuroscience*. 2002; 15:120–132. [PubMed: 11860512]
15. Buck K, Voehringer P, Ferger B. The alpha(2) adrenoceptor antagonist idazoxan alleviates L-DOPA-induced dyskinesia by reduction of striatal dopamine levels: an in vivo microdialysis study in 6-hydroxydopamine-lesioned rats. *Journal of neurochemistry*. 2010; 112:444–452. [PubMed: 19895663]
16. Barnum CJ, Bhide N, Lindenbach D, Surrena MA, Goldenberg AA, Tignor S, et al. Effects of noradrenergic denervation on L-DOPA-induced dyskinesia and its treatment by alpha- and beta-adrenergic receptor antagonists in hemiparkinsonian rats. *Pharmacology, biochemistry, and behavior*. 2012; 100:607–615.
17. Savola JM, Hill M, Engstrom M, Merivuori H, Wurster S, McGuire SG, et al. Fipamezole (JP-1730) is a potent alpha2 adrenergic receptor antagonist that reduces levodopa-induced dyskinesia in the MPTP-lesioned primate model of Parkinson's disease. *Movement disorders : official journal of the Movement Disorder Society*. 2003; 18:872–883. [PubMed: 12889076]
18. Grondin R, Hadj Tahar A, Doan VD, Ladure P, dard PJ. Noradrenoceptor antagonism with idazoxan improves l -dopa-induced dyskinesias in MPTP monkeys. *Naunyn-Schmiedeberg's Archives of Pharmacology*. 2000; 361:181–186. x000E.
19. Rascol O, Arnulf I, Peyro-Saint Paul H, Brefel-Courbon C, Vidailhet M, Thalamas C, et al. Idazoxan, an alpha-2 antagonist, and L-DOPA-induced dyskinesias in patients with Parkinson's disease. *Movement disorders : official journal of the Movement Disorder Society*. 2001; 16:708–713. [PubMed: 11481696]
20. Johnston TH, Fox SH, Piggott MJ, Savola JM, Brotchie JM. The alpha(2) adrenergic antagonist fipamezole improves quality of levodopa action in Parkinsonian primates. *Movement disorders : official journal of the Movement Disorder Society*. 2010; 25:2084–2093. [PubMed: 20824735]
21. Henry B, Fox SH, Peggs D, Crossman AR, Brotchie JM. The alpha2-adrenergic receptor antagonist idazoxan reduces dyskinesia and enhances anti-parkinsonian actions of L-DOPA in the MPTP-lesioned primate model of Parkinson's disease. *Movement disorders : official journal of the Movement Disorder Society*. 1999; 14:744–753. [PubMed: 10495035]
22. Rosin DL, Talley EM, Lee A, Stornetta RL, Gaylinn BD, Guyenet PG, et al. Distribution of alpha 2C-adrenergic receptor-like immunoreactivity in the rat central nervous system. *The Journal of comparative neurology*. 1996; 372:31.

23. Alachkar A, Brotchie JM, Jones OT. Changes in the mRNA levels of alpha(2A) and alpha(2C) adrenergic receptors in rat models of Parkinson's disease and L-DOPA-induced dyskinesia. *J Mol Neurosci*. 2011; 46:145–152. [PubMed: 21562737]
24. Fox SH, Henry B, Hill MP, Peggs D, Crossman AR, Brotchie JM. Neural mechanisms underlying peak-dose dyskinesia induced by levodopa and apomorphine are distinct: evidence from the effects of the alpha(2) adrenoceptor antagonist idazoxan. *Movement disorders : official journal of the Movement Disorder Society*. 2001; 16:642–650. [PubMed: 11481687]
25. Miguelez C, Aristieta A, Cenci MA, Ugedo L. The locus coeruleus is directly implicated in L-DOPA induced dyskinesia in Parkinsonian rats: An electrophysiological and behavioural study. *PLoS ONE*. 2011; 6:1–12.
26. Norenberg W, Schoffel E, Szabo B, Starke K. Subtype determination of soma-dendritic alpha2-autoreceptors in slices of rat locus coeruleus. *Naunyn-Schmiedeberg's Archives of Pharmacology*. 1997; 356:7.
27. Yavich L, Lappalainen R, Sirvio J, Haapalinna A, MacDonald E. Alpha2-adrenergic control of dopamine overflow and metabolism in mouse striatum. *European journal of pharmacology*. 1997; 339:113–119. [PubMed: 9473124]
28. Yavich L, Sirvio J, Haapalinna A, Ylinen A, Mannisto PT. Atipamezole, an alpha2-adrenoceptor antagonist, augments the effects of L-DOPA on evoked dopamine release in rat striatum. *European journal of pharmacology*. 2003; 462:83–89. [PubMed: 12591099]
29. Nutt, DJ.; Lalties, MD.; Hudson, AL. The effects of alpha2-adrenoceptor antagonists on extracellular dopamine concentrations in rat striatum. In: Briley, M.; Marien, M., editors. *Noradrenergic Mechanisms in Parkinson's Disease*. CRC Press; 1994. p. 159-172.
30. Bucheler MM, Hadamek K, Hein L. Two alpha(2)-adrenergic receptor subtypes, alpha(2A) and alpha(2C), inhibit transmitter release in the brain of gene-targeted mice. *Neuroscience*. 2002; 109:819–826. [PubMed: 11927164]
31. Pertovaara A, Haapalinna A, Sirvio J, Virtanen R. Pharmacological properties, central nervous system effects, and potential therapeutic applications of atipamezole, a selective alpha2-adrenoceptor antagonist. *CNS drug reviews*. 2005; 11:273–288. [PubMed: 16389294]
32. Cenci MA. Dopamine dysregulation of movement control in L-DOPA-induced dyskinesia. *Trends in neurosciences*. 2007; 30:236–243. [PubMed: 17400300]
33. Chang JW, Wachtel SR, Young D, Kang UJ. Biochemical and anatomical characterization of forepaw adjusting steps in rat models of Parkinson's disease: studies on medial forebrain bundle and striatal lesions. *Neuroscience*. 1999; 88:617–628. [PubMed: 10197780]
34. Dupre KB, Eskow KL, Barnum CJ, Bishop C. Striatal 5-HT1A receptor stimulation reduces D1 receptor-induced dyskinesia and improves movement in the hemiparkinsonian rat. *Neuropharmacology*. 2008; 55:1321–1328. [PubMed: 18824001]
35. Bishop C, Krolewski DM, Eskow KL, Barnum CJ, Dupre KB, Deak T, et al. Contribution of the striatum to the effects of 5-HT1A receptor stimulation in L-DOPA-treated hemiparkinsonian rats. *Journal of neuroscience research*. 2009; 87:1645–1658. [PubMed: 19115412]
36. Kilpatrick IC, Jones MW, Phillipson OT. A semiautomated analysis method for catecholamines, indoleamines, and some prominent metabolites in microdissected regions of the nervous system: an isocratic HPLC technique employing coulometric detection and minimal sample preparation. *Journal of neurochemistry*. 1986; 46:12. [PubMed: 2866232]
37. Ostock CY, Dupre KB, Jaunarajs KL, Walters H, George J, Krolewski D, et al. Role of the primary motor cortex in L-Dopa-induced dyskinesia and its modulation by 5-HT1A receptor stimulation. *Neuropharmacology*. 2011; 61:753–760. [PubMed: 21635907]
38. Colosimo C, Craus A. Noradrenergic drugs for levodopa-induced dyskinesia. *Clin Neuropharmacol*. 2003; 26:299–305. [PubMed: 14646609]
39. Buck K, Ferger B. The selective alpha1 adrenoceptor antagonist HEAT reduces L-DOPA-induced dyskinesia in a rat model of Parkinson's disease. *Synapse*. 2010; 64:117–126. [PubMed: 19771592]
40. Visanji NP, Fox SH, Johnston TH, Millan MJ, Brotchie JM. Alpha1-adrenoceptors mediate dihydroxyphenylalanine-induced activity in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-

- lesioned macaques. *The Journal of pharmacology and experimental therapeutics*. 2009; 328:276–283. [PubMed: 18955589]
41. Manson AJ, Iakovidou E, Lees AJ. Idazoxan is ineffective for levodopa-induced dyskinesias in Parkinson's disease. *Movement disorders : official journal of the Movement Disorder Society*. 2000; 15:2. [PubMed: 10755265]
 42. Lewitt PA, Hauser RA, Lu M, Nicholas AP, Weiner W, Coppard N, et al. Randomized clinical trial of fipamezole for dyskinesia in Parkinson's disease (FJORD study). *Neurology*. 2012; 79:163–169. [PubMed: 22744665]
 43. Convents A, De Keyser J, De Backer JP, Vauguelin G. [3H]rauwolscine labels alpha 2-adrenoceptors and 5-HT1A receptors in human cerebral cortex. *European journal of pharmacology*. 1989; 159:4.
 44. Millan MJ, Newman-Tancredi A, Audinot V, Cussac D, Lejeune F, Nicholas JP, et al. Agonist and antagonist actions of yohimbine as compared to fluparoxan at adrenergic receptors (AR)s serotonin (5HT)(1A), 5-HT(1B), 5-HT(1D), dopamine D(2), D(3) receptors Significance for the modulation of frontocortical monoaminergic transmission and depressive states. *Synapse*. 2000; 35:79–95. [PubMed: 10611634]
 45. Newman-Tancredi A, Nicolas JO, Audinot V, Gavaudan S, Verrielle L, Touzard M, et al. Actions of alpha2-adrenoceptor ligands at alpha2A and 5-HT1A receptors: the antagonist, atipamezole and the agonist, dexmedetomidine, are highly selective for alpha2A adrenoceptors. *Naunyn-Schmiedeberg's Arch of Pharmacol*. 1998; 358:197–206. [PubMed: 9750005]
 46. Scatton B, Zivkovic B, Dedek J. Antidopaminergic properties of yohimbine. *The Journal of pharmacology and experimental therapeutics*. 1980; 215:494–499. [PubMed: 7192314]
 47. Taylor JL, Bishop C, Walker PD. Dopamine D1 and D2 receptor contributions to L-DOPA-induced dyskinesia in the dopamine-depleted rat. *Pharmacology, biochemistry, and behavior*. 2005; 81:887–893.
 48. Carta AR, Frau L, Pinna A, Morelli M. Dyskinetic potential of dopamine agonists is associated with different striatonigral/striatopallidal zif-268 expression. *Experimental neurology*. 2010; 224:395–402. [PubMed: 20452347]
 49. Huot P, Brotchie JM. 5-HT(1A) receptor stimulation and L-DOPA-induced dyskinesia in Parkinson's disease: bridging the gap between serotonergic and glutamatergic mechanisms. *Experimental neurology*. 2011; 231:195–198. [PubMed: 21819982]
 50. Koprich JB, Huot P, Fox SH, Jarvie K, Lang AE, Seeman P, et al. The effects of fast-off-D2 receptor antagonism on L-DOPA-induced dyskinesia and psychosis in parkinsonian macaques. *Progress in neuro-psychopharmacology & biological psychiatry*. 2013; 43:151–156. [PubMed: 23306217]
 51. Eskow KL, Gupta V, Alam S, Park JY, Bishop C. The partial 5-HT(1A) agonist bupirone reduces the expression and development of L-DOPA-induced dyskinesia in rats and improves L-DOPA efficacy. *Pharmacology, biochemistry, and behavior*. 2007; 87:306–314.
 52. Ernsberger P, Meeley MP, Mann JJ, Reis DJ. Clonidine binds to imidazole binding sites as well as alpha 2-adrenoceptors in the ventrolateral medulla. *European journal of pharmacology*. 1987; 134:1–13. [PubMed: 3030779]
 53. Couprie I, Lachaud V, Podevin RA, Koenig E, Parini A. Different affinities of alpha 2-agonists for imidazoline and alpha 2-adrenergic receptors. *American journal of hypertension*. 1989; 2:468–470. [PubMed: 2569318]
 54. Haapalinna A, Leino T, Heinonen E. The alpha 2-adrenoceptor antagonist atipamezole potentiates anti-Parkinsonian effects and can reduce the adverse cardiovascular effects of dopaminergic drugs in rats. *Naunyn Schmiedeberg's Arch Pharmacol*. 2003; 368:342–351. [PubMed: 14566451]
 55. Mavridis M, Colpaert F, Millan MJ. Differential modulation of (+)-amphetamine induced rotations in unilateral SN lesioned rats by alpha 1 as compared to alpha 2 agonists and antagonists. *Brain Res*. 1991:562.
 56. Juhila J, Haapalinna A, Sirvio J, Sallinen J, Honkanen A, Korpi ER, et al. The alpha2-adrenoceptor antagonist atipamezole reduces the development and expression of d-amphetamine-induced behavioural sensitization. *Naunyn Schmiedeberg's Arch Pharmacol*. 2003; 367:274–280. [PubMed: 12644900]

57. Huotari M, Kukkonen K, Liikka N, Potasev T, Raasmaja A, Mannisto PT. Effects of histamine H(3)-ligands on the levodopa-induced turning behavior of hemiparkinsonian rats. *Parkinsonism & related disorders*. 2000; 6:159–164. [PubMed: 10817955]
58. Chopin P, Colpaert F, Marien M. Effects of alpha-2 adrenoceptor agonists and antagonists on circling behavior in rats with unilateral 6-hydroxydopamine lesions of the nigrostriatal pathway. *The Journal of pharmacology and experimental therapeutics*. 1999; 288:798–804. [PubMed: 9918591]
59. Lee A, Rosin DL, Van Bockstaele EJ. alpha2A-adrenergic receptors in the rat nucleus locus coeruleus: subcellular localization in catecholaminergic dendrites, astrocytes, and presynaptic axon terminals. *Brain Res*. 1998; 795:157–169. [PubMed: 9622618]
60. Lee A, Rosin DL, Van Bockstaele EJ. Ultrastructural evidence for prominent postsynaptic localization of alpha2C-adrenergic receptors in catecholaminergic dendrites in the rat nucleus locus coeruleus. *The Journal of comparative neurology*. 1998; 394:218–229. [PubMed: 9552127]
61. MacDonald E, Scheinin M. Distribution and pharmacology of alpha 2-adrenoceptors in the central nervous system. *Journal of physiology and pharmacology : an official journal of the Polish Physiological Society*. 1995; 46:241–258. [PubMed: 8527807]
62. Nicholas AP, Pieribone VA, Hokfelt T. Cellular localization of messenger RNA for beta-1 and beta-2 adrenergic receptors in rat brain: An in situ hybridization study. *Neuroscience*. 1993; 56:17.
63. Holmberg M, Scheinin M, Kurose H, Miettinen R. Adrenergic alpha2C-receptors reside in rat striatal GABAergic projection neurons: comparison of radioligand binding and immunohistochemistry. *Neuroscience*. 1999; 93:1323–1333. [PubMed: 10501456]
64. Scheinin M, Lomasney JW, Hayden-Hixson DM, Schambra UB, Caron MG, Lefkowitz RJ, et al. Distribution of alpha 2-adrenergic receptor subtype gene expression in rat brain. *Brain research Molecular brain research*. 1994; 21:133–149. [PubMed: 8164514]
65. Buck K, Ferger B. Comparison of intrastriatal administration of noradrenaline and L-DOPA on dyskinetic movements: a bilateral reverse in vivo microdialysis study in 6-hydroxydopamine-lesioned rats. *Neuroscience*. 2009; 159:16–20. [PubMed: 19146929]
66. Lapiz MD, Zhao Z, Bondi CO, O'Donnell JM, Morilak DA. Blockade of autoreceptor-mediated inhibition of norepinephrine release by atipamezole is maintained after chronic reuptake inhibition. *Int J Neuropsychopharmacol*. 2007; 10:827–833. [PubMed: 17697440]
67. Svensson TH, Bunney BS, Aghajanian GK. Inhibition of both noradrenergic and serotonergic neurons in brain by the alpha-adrenergic agonist clonidine. *Brain Res*. 1975; 92:291–306. [PubMed: 1174954]
68. Langer SZ. Presynaptic regulation of catecholamine release. *Biochemical pharmacology*. 1974; 23:1793–1800. [PubMed: 4617579]
69. Hudson AL, Robinson ES, Lallies MD, Tyacke RJ, Jackson HC, Nutt DJ. In vitro and in vivo approaches to the characterization of the alpha2-adrenoceptor. *J Auton Pharmacol*. 1999; 19:311–320. [PubMed: 10961736]
70. Hertel P, Nomikos GG, Svensson TH. Idazoxan preferentially increases dopamine output in the rat medialprefrontal cortex at the nerve terminal level. *European journal of pharmacology*. 1999; 371:153–158. [PubMed: 10357252]
71. Trendelenburg A, Starke K, Limberger N. Presynaptic alpha 2A-adrenoceptors inhibit the release of endogenous dopamine in rabbit caudate nucleus slices. *Naunyn-Schmiedeberg's Arch of Pharmacol*. 1994; 350:9.
72. Arai A, Tomiyama M, Kannari K, Kimura T, Suzuki C, Watanabe M, et al. Reuptake of L-DOPA-derived extracellular DA in the striatum of a rodent model of Parkinson's disease via norepinephrine transporter. *Synapse*. 2008; 62:632–635. [PubMed: 18509855]
73. Chotibut T, Fields V, Salvatore MF. Norepinephrine transporter inhibition with desipramine exacerbates L-DOPA-induced dyskinesia: role for synaptic dopamine regulation in denervated nigrostriatal terminals. *Molecular pharmacology*. 2014; 86:675–685. [PubMed: 25208966]
74. Anden N, Grabowska M. Pharmacological evidence for a stimulation of dopamine neurons by noradrenaline neurons in the brain. *European journal of pharmacology*. 1976; 39:275–282. [PubMed: 185062]

75. Fujimoto S, Sasa M, Takaori S. Inhibition from locus coeruleus of caudate neurons activated by nigral stimulation. *Brain Res Bull.* 1981; 6:8.
76. Tanaka H, Kannari K, Maeda T, Tomiyama M, Suda T, Matsunaga M. Role of serotonergic neurons in L-DOPA-derived extracellular dopamine in the striatum of 6-OHDA-lesioned rats. *Neuroreport.* 1999; 10:631–634. [PubMed: 10208602]
77. Carta M, Carlsson T, Kirik D, Bjorklund A. Dopamine released from 5-HT terminals is the cause of L-DOPA-induced dyskinesia in parkinsonian rats. *Brain : a journal of neurology.* 2007; 130:1819–1833. [PubMed: 17452372]
78. Navailles S, Bioulac B, Gross C, De Deurwaerdere P. Chronic L-DOPA therapy alters central serotonergic function and L-DOPA-induced dopamine release in a region-dependent manner in a rat model of Parkinson's disease. *Neurobiology of disease.* 2011; 41:585–590. [PubMed: 21092759]
79. Talley EM, Rosin DL, Lee A, Guyenet PG, Lynch KR. Distribution of alpha 2A-adrenergic receptor-like immunoreactivity in the rat central nervous system. *The Journal of comparative neurology.* 1996; 372:24.
80. Day HE, Campeau S, Watson SJ Jr, Akil H. Distribution of alpha 1a-, alpha 1b- and alpha 1d-adrenergic receptor mRNA in the rat brain and spinal cord. *Journal of chemical neuroanatomy.* 1997; 13:115–139. [PubMed: 9285356]
81. Baraban JM, Aghajanian GK. Suppression of firing activity of 5-HT neurons in the dorsal raphe by alpha-adrenoceptor antagonists. *Neuropharmacology.* 1980; 19:355–363. [PubMed: 6104308]
82. Haddjeri N, Lavoie N, Blier P. Electrophysiological evidence for the tonic activation of 5-HT(1A) autoreceptors in the rat dorsal raphe nucleus. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology.* 2004; 29:1800–1806. [PubMed: 15127086]

Highlights

- Clonidine suppressed L-DOPA-induced motor activity and dyskinesia in rats.
- Atipamezole enhanced L-DOPA-induced motor activity and dyskinesia in rats.
- Locus coeruleus $\alpha 2$ -noradrenergic receptors regulated L-dopa-induced dyskinesia.

Experimental Timeline

Figure 1. Diagram of the experimental timeline and design

Following recovery from surgery, all rats were treated with L-DOPA (12 mg/kg, sc) in order to produce maximal behavioral responses to future L-DOPA injections [37]. On the 7th day of L-DOPA (12 mg/kg, sc) treatment, rats were rated for AIMs and only those that reached dyskinetic criteria (AIMs scores > 30) were retained for further testing. Behavioral testing to determine the effects of systemic (experiment 1; Figure 1A) or intra-LC (experiment 2; Figure 1B) clonidine (n = 8) or atipamezole (n = 11) on L-DOPA mediated behaviors began 4 days later. Each subsequent behavioral testing day was separated by 2–4 days, and only 1 behavioral test was performed each day. Following completion of all behavioral assays, rats were killed off treatment for neurochemical verification of lesion using HPLC-ED.

Figure 2. Effect of Systemic $\alpha 2R$ treatment on ALO AIM and Rotations

Rats ($n = 10$) were treated with clonidine (0, 0.01, 0.1 mg/kg, ip; C0-LD, C0.01-LD, C0.1-LD) or atipamezole (0, 0.3, 1.0 mg/kg, sc; A0-LD, A0.3-LD, A1.0-LD) followed 30 min later by L-DOPA 4 mg/kg (top panels) or 6 mg/kg (bottom panels) using a counterbalanced, within-subjects design. Graphs depict the timecourse of ALO AIMs (expressed as medians $\pm$ median absolute deviation, MAD; Clonidine: A, C; Atipamezole E, G) and rotations (expressed as means $\pm$ standard error of the mean, SEM; Clonidine: B, D; Atipamezole F, H). Insets represent total ALO AIMs or rotations summed over the 3 h testing period.

Treatment effects were analyzed with Friedman tests for AIMs and two-way repeated measures ANOVAs for rotations. Post-hoc comparisons denote significant differences between treatments at the time-points. Clonidine: * $p < 0.05$ C0.1-LD vs. C0-LD; + $p < 0.05$ C0.01-LD vs. C0-LD; ^ $p < 0.05$ C0.1-LD vs. C0.01-LD. Atipamezole: * $p < 0.05$ A1.0-LD vs. A0-LD; + $p < 0.05$ A0.3-LD vs. A0-LD; ^ $p < 0.05$ A1.0-LD-A0.3-LD

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Figure 3. Effect of systemic α_2 R treatment on L-DOPA-induced improvement of stepping
 Rats ($n = 8$) were injected with the clonidine (0, 0.1 mg/kg, ip) or atipamezole (0, 1.0 mg/kg, sc) followed 30 min later by L-DOPA (0, 4, 6 mg/kg, sc) using a counterbalanced, within-subjects design. The FAS test was performed 60 min after L-DOPA treatment. Stepping performance is expressed as mean percent ($\pm$ standard error of the mean, SEM) of intact forehand (A, D) or backhand (B, C) stepping. Treatment effects were determined by one-factor repeated-measures ANOVAs for each metric. Post hoc comparisons denote significant differences between treatments. Clonidine: * $p < 0.05$ vs. C0-LD0; + $p < 0.05$ vs. C0-LD4. Atipamezole: * $p < 0.05$ vs. A0-LD0; + $p < 0.05$ vs. A0-LD4.

Figure 4. Effect of systemic α_2 R treatment on L-DOPA's motor-facilitating effects

Rats ($n = 8$) were injected with clonidine (0, 0.1 mg/kg, ip) or atipamezole (0, 1.0 mg/kg, sc) followed 30 min later by L-DOPA (0, 4, 6 mg/kg, sc) using a counterbalanced, within-subjects design. Immediately following L-DOPA treatment, rats were individually placed into locomotor activity chambers. Data from three separate metrics of motor activity, distance traveled (clonidine: A; atipamezole: D), vertical activity (clonidine: B; atipamezole: E), and stereotypy count (clonidine: C; atipamezole: F) were recorded across 3 1 h time bins. Bars depict the mean (+ standard error of the mean, SEM) activity measure for each

treatment at each time point. Treatment and time effects were analyzed using 6(treatment) $\times$ 3(time) within-subjects repeated measures ANOVAs for each behavior. Post hoc comparisons denote significant differences between treatments at the time-points indicated. Clonidine: * $p < 0.05$ vs. C0-LD0; + $p < 0.05$ vs. C0-LD4; ^ $p < 0.05$ vs. C0-LD6. Atipamezole: * $p < 0.05$ vs. A0-LD0; + $p < 0.05$ vs. A0-LD4; ^ $p < 0.05$ vs. A0-LD6

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Figure 5. The effect of intra-LC α_2R treatment on LID

Schematic representation of coronal rat brain section containing the LC adapted from Paxinos and Watson (Bregma: -10.04 mm) and a representative cresyl violet-stained LC image portraying typical injector placement is displayed (A). Relevant anatomical structures: Cerebellum (Cer), Fourth ventricle (4V), Mesencephalic trigeminal nucleus (Me5). The effects of intra-LC clonidine or atipamezole on ALO AIMs (clonidine: B; atipamezole: C) and rotations (clonidine: D; atipamezole: E) were assessed in 2 separate groups of rats (clonidine treated: $n = 8$; atipamezole treated ($n = 11$)). Clonidine ($0, 2, 20$ μg) or atipamezole ($0, 1.5, 15.0$ μg) was injected into the LC followed by systemic injection of L-DOPA (clonidine treated: $0, 6$ mg/kg; atipamezole: $0, 4$ mg/kg, sc). Graphs depict the timecourse of ALO AIMs (expressed as medians $\pm$ median absolute deviation, MAD; A) and rotations (expressed as means $\pm$ standard error of the mean, SEM; B). Insets represent total ALO AIMs and rotations summed over the 3 h testing period. Treatment effects were analyzed with Friedman tests for AIMs and two-way repeated measures ANOVAs for rotations. Post-hoc comparisons denote significant differences between treatments at the time-points indicated. Clonidine: * $p < 0.05$ C0-LD6 vs. C20-LD6; + $p < 0.05$ C0-LD6 vs. C2-LD6; ^ CL2-LD6 vs. CL20-LD6. Atipamezole: * $p < 0.05$, A0-LD4 vs. A15.0-LD4; + $p < 0.05$, A0-LD4 vs. A15.0-LD4; ^ $p < 0.05$, A0-LD0 vs. A15.0-LD0.

Table 1

Drug Treatment Schedule

Clonidine (Intra LC- Systemic) n = 8		Atipamezole (Intra LC- Systemic) n = 11	
Intra-LC Treatment (dose)	Systemic Treatment (dose)	Intra-LC treatment (dose)	Systemic Treatment (dose)
--	--	Atipamezole (0.0 µg)	L-DOPA (0 mg/kg) (A0-LD0)
Clonidine (0.0 µg)	L-DOPA (6 mg/kg)	Atipamezole (0.0 µg)	L-DOPA (4 mg/kg) (A0-LD4)
Clonidine (2.0 µg)	L-DOPA (6 mg/kg)	Atipamezole (1.5 µg)	L-DOPA (4 mg/kg) (A1.5-LD4)
Clonidine (20.0 µg)	L-DOPA (6 mg/kg)	Atipamezole (15.0 µg)	L-DOPA (4 mg/kg) (A15.0-LD4)
Clonidine (20.0 µg)	L-DOPA (0 mg/kg)	Atipamezole (15.0 µg)	L-DOPA (0 mg/kg) (A15.0-LD4)

Table 2

Effects of unilateral MFB lesion on concentrations of striatal NE, DA, and DOPAC

Hemisphere	NE	DA	DOPAC
Lesion (left)	7.83 ± 1.38*	55.37 ± 13.06*	28.94 ± 4.38*
Intact (Right)	39.32 ± 6.80	4544.93 ± 449.03	1677.81 ± 173.77

Values represent pictogram monoamine or metabolite per milligram protein (mean ± standard error of the mean).

*
p < 0.05 vs. intact hemisphere