

Serotonin 5-HT_{2A} Receptors Underlie Increased Motor Behaviors Induced in Dopamine-Depleted Rats by Intrastratial 5-HT_{2A/2C} Agonism

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ABSTRACT

Gene expression studies have suggested that dopamine (DA) depletion increases the sensitivity of striatal direct pathway neurons to the effects of serotonin (5-HT) via the 5-HT₂ receptor. The present study examined the possible influence(s) of 5-HT_{2A} or 5-HT_{2C} receptor-mediated signaling locally within the striatum on motor behavior triggered by 5-HT₂ receptor agonism in the neonatal DA-depleted rat. Male Sprague-Dawley rats were treated with 6-hydroxydopamine (6-OHDA; 60 μg in 5 μl per lateral ventricle) on postnatal day 3 to achieve near-total DA depletion bilaterally. Sixty days later, sham-operated (saline-injected) or 6-OHDA-treated rats were challenged with the 5-HT_{2A/2C} agonist DOI [(±)-1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane] or saline either by systemic treatment or bilateral intrastratial infusion. Motor behavior was quantified for 60 min after agonist injection using computerized activity monitors. Systemic DOI treatment (0.2 or 2.0 mg/kg i.p.) was more effective in inducing motor activity in

the DA-depleted group compared with intact controls. Intrastratial DOI infusion (1.0 or 10.0 μg/side) also produced a significant rise in motor activity in the DA-depleted group during the 30- to 60-min period of behavioral analysis but did not influence behavior in intact animals. The effects of intrastratial DOI infusion were blocked by intrastratial coinjection of the 5-HT₂ antagonist ketanserin (1.0 μg) and the 5-HT_{2A}-preferring antagonist M100907 [(R)-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidinemethanol; 1.0 μg] but not the 5-HT_{2C}-preferring antagonist RS102221 [8-[5-(2,4-dimethoxy-5-(4-trifluoromethylsulfo-amido)phenyl-5-oxopentyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione; 1.0 μg]. Such results support the hypothesis that 5-HT_{2A} receptor-mediated signaling events are strengthened within the striatum under conditions of DA depletion to provide a more potent regulation of motor activity.

A loss of dopamine (DA) transmission to the rodent striatum during early postnatal development results in a compensatory increase in serotonin (5-HT) innervation to the dorsal striatum (Breese et al., 1984; Stachowiak et al., 1984). The "5-HT hyperinnervation" phenomenon arises from raphe-striatal axons that normally provide heavier 5-HT innervation to the caudal striatum but sprout collaterals to the more sparsely innervated rostral striatum in response to neonatal

DA depletion (Berger et al., 1985; Snyder et al., 1986; Luthman et al., 1987; Towle et al., 1989). Although this plasticity leads to a doubling of [³H]5-HT-labeled axonal varicosities (Mrini et al., 1995), they do not form new synapses in significant numbers (Descarries et al., 1992). Rather, sprouted axons appear to accumulate significant amounts of 5-HT (Molina-Holgado et al., 1994) so that extracellular 5-HT levels are tripled under stimulatory release conditions (Jackson and Abercrombie, 1992).

Several studies indicate that 5-HT_{2A} receptors are positioned to mediate the influences of enhanced 5-HT signaling in the DA-depleted striatum. First, 5-HT release agents and 5-HT₂ receptor agonists gain potency in inducing striatal preprotachykinin (PPT; encodes substance P and neurokinin

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ABBREVIATIONS: DA, dopamine; 5-HT, 5-hydroxytryptamine (serotonin); PPT, preprotachykinin; DOI, (±)-1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane; 6-OHDA, 6-hydroxydopamine; M100907, (R)-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidinemethanol; RS102221, 8-[5-(2,4-dimethoxy-5-(4-trifluoromethylsulfo-amido)phenyl-5-oxopentyl)-1,3,8-triazaspiro[4.5]decane-2,4-dione; ANOVA, analysis of variance; DOPAC, 3,4-dihydroxyphenylacetic acid; 5-HIAA, 5-hydroxyindole-3-acetic acid.

A gene expression within the direct striatal output pathway) under conditions of DA depletion (Gresch and Walker, 1999; Basura and Walker, 2000, 2001). Second, 5-HT₂ receptor-mediated signaling appears to contribute to spontaneous hyperactivity observed in neonatal DA-depleted rats (Luthman et al., 1991) and also fosters D₁ receptor-mediated hyperlocomotive behaviors following adult DA lesions (Bishop et al., 2003; Bishop and Walker, 2003). Third, similar to the 5-HT sprouting phenomenon, the 5-HT_{2A} receptor is more heavily expressed within the dorsal striatum following neonatal DA depletion (Radja et al., 1993; Laprade et al., 1996; Basura and Walker, 2001). These convergent results have led us to hypothesize that 5-HT_{2A} receptor stimulation may underlie hyperlocomotive behaviors in the DA-depleted rat.

The present study examined the effects of 5-HT₂ receptor agonism on motor behaviors expressed in adult rats that had received bilateral DA lesions as neonates. Although 5-HT₂ receptor agonists such as DOI [(±)-1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane] produce minimal effects on locomotor behavior in the intact rat (Koskinen et al., 2000), we predicted that systemic DOI treatments would increase locomotor behaviors in 6-OHDA-treated rats since 5-HT₂ receptor stimulation has previously been shown to gain potency in regulating direct pathway gene expression (Gresch and Walker, 1999; Basura and Walker, 2001). To limit the effects of DOI to the striatal region that demonstrates prominent 5-HT_{2A} receptor plasticity, we measured behavior in response to local DOI infusion into the dorsal striatum of intact and DA-depleted groups. These latter experiments also included the coinfusion of selective antagonists M100907 (Johnson et al., 1996; Kehne et al., 1996) and RS102221 (Bonhaus et al., 1997) to discern the effects of local striatal 5-HT_{2A} versus 5-HT_{2C} receptor activation, respectively, on DOI-induced motor activity. The results obtained suggest that 5-HT_{2A} receptor plasticity within the dorsal striatum is functionally significant and may influence motor control mechanisms of the DA-depleted rodent.

Materials and Methods

Animals. Male Sprague-Dawley rat pups (Charles River Laboratories, Inc., Wilmington, MA) were used and maintained in strict accordance with the guidelines of the Institutional Animal Care and Use Committee of Wayne State University and the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication no. 85-23, revised 1996). Rat pups arrived on postnatal day 2 and were initially group-housed ($n = 12/\text{dam}$) in plastic cages (22 cm high, 45 cm deep, and 23 cm wide) within a temperature-controlled colony room (22–23°C) illuminated on a 12-h light/dark cycle (lights on 7:00 AM). Once rats were 3 weeks old, they were weaned from the dams, group-housed (4–5/cage), and fed standard lab chow (Rodent Diet 5001; LabDiet, Brentwood, MO) and water, which were available throughout the remainder of the experiment.

6-OHDA Lesions and Cannulae Implantation Surgeries. Bilateral intracerebroventricular 6-OHDA lesions were performed as reported previously (Basura and Walker, 1999). On postnatal day 3, rat pups were anesthetized to effect by hypothermia (body immersion in a foil chamber cooled in wet ice), and a midsagittal incision was made on the dorsal scalp to reveal the skull sutures and obtain reference coordinates from bregma. Each rat received a bilateral infusion of 6-OHDA (60 µg; Sigma-Aldrich, St. Louis, MO) or its vehicle solution of saline containing 0.1% ascorbic acid (Sigma-Aldrich) at a rate of 5 µl over 30 s into each ventricle (anterior/posterior, 0.0 mm; medial/lateral, ±1.0 mm; dorsal/ventral, –2.5 mm

relative to bregma) using a 10-µl syringe equipped with a 30-gauge needle. Pups were placed under a heat lamp for recovery prior to being returned to the mother rat. All rats received subcutaneous injections of desipramine (25 mg/kg; Sigma-Aldrich) 30 min prior to surgery to protect norepinephrine neurons. Sixty days following i.c.v. lesions, rats were anesthetized with an i.p. injection of ketamine (90 mg/kg; Lloyd Laboratories, Shenandoah, IA) and xylazine (15 mg/kg; Lloyd Laboratories) mixture and implanted bilaterally with chronic 22-gauge intracranial guide cannulae (C313G/SPC; Plastics One Inc., Roanoke, VA). With the incisor bar positioned 3.3 mm below the interaural line, cannulae were positioned above the dorsal striatum bilaterally using the coordinates anterior/posterior, +0.4 mm; medial/lateral, ±2.9 mm; and dorsal/ventral, –3.6 mm relative to bregma (Paxinos and Watson, 1998). Cannulae were fixed in place using liquid and powder dental acrylic (Lang Dental, Wheeling, IL). At the completion of surgery, guide cannulae were fitted with 28 gauge inner stylets (Plastics One) to maintain patency.

Pharmacological Treatments. The following 5-HT₂ receptor agents were used in this study: DOI (Sigma-Aldrich), ketanserin (Sigma-Aldrich), M100907 (synthesized as described by Ullrich and Rice, 2000), and RS102221 (Tocris Cookson Inc., Ellisville, MO). At least 1 week after cannulation, rats were randomly divided into treatment groups and assigned to receive a regimen of systemic and/or bilateral intrastriatal drug infusions on separate testing occasions. Intact control and DA-lesioned rats ($n = 20$) received an i.p. injection of saline vehicle or the 5-HT_{2A/2C} agonist DOI using 0.2 or 2.0 mg/kg doses in a repeated-measures design with groups counterbalanced to avoid order effects. For local manipulations, rats ($n = 50$) were tested for locomotor effects elicited by intrastriatal drug infusions of saline or DOI (1.0 or 10 µg) at a volume of 0.8 µl/side. To determine whether the effects of DOI were more likely to be mediated by 5-HT_{2A} or 5-HT_{2C} receptors, these rats were then tested for responses to vehicle (10% dimethyl sulfoxide in saline), 1.0 µg of DOI, 1.0 µg of the 5-HT₂ antagonist ketanserin, 1.0 µg of the 5-HT_{2C}-preferring antagonist RS102221, 1.0 µg of the 5-HT_{2A}-preferring antagonist M100907, or DOI coinified with each of these antagonists (1.0 µg of agonist + 1.0 µg of antagonist) at a volume of 0.8 µl/side in a counterbalanced design. The number of animals per group ranged from 7 to 10 rats. No rat was tested more than three times, and at least 3 days separated each testing session.

Behavioral Procedure. Locomotor activity monitoring was conducted in six identical acrylic chambers measuring 40 cm long, 40 cm wide, and 30 cm high (AccuScan Instruments, Inc., Columbus, OH). Each chamber was surrounded by a 15 × 15 infrared photocell array interfaced with a computer that ran the Versamax and Versadat programs (AccuScan Instruments, Inc.). These programs tabulated and processed a number of variables related to locomotor behavior. For the present experiment, the variables collected over twenty 3-min periods were horizontal activity (beam breaks counted across the photocell array), distance traveled (in centimeters), movement bouts (number of times a rat ended and then initiated another movement period), stereotypy activity (beam breaks counted at the same photocell array), and stereotypy bouts (number of times a rat ended and then initiated another stereotypy period). Prior to experimentation, rats were acclimated to the behavioral procedure at least three times. All testing occurred between 8:30 AM and 12:30 PM. On testing days, rats were placed in the activity chambers for 30 min before injections commenced. During injections, rats were lightly restrained with a towel, and 15-mm 30-gauge injectors (Plastics One) were slowly lowered to extend 1 mm past the end of the guide cannulae. Once injectors were in place, drug was infused at a rate of 0.63 µl/min by a microinfusion pump (Harvard Apparatus Inc., Holliston, MA) that held two 50-µl Hamilton syringes attached to plastic tubing (PE20 Tygon tubing; Plastics One) and the injectors. Following infusions, injectors remained in place for 30 s. Immediately after the injectors were removed, the pump was restarted, and injectors were inspected for fluid expulsion.

Tissue Dissection and Cryostat Sectioning. When experiments were completed, rats were sacrificed by decapitation, and brains were immediately removed and dissected. Tissue from 20 randomly selected rats (10 control and 10 6-OHDA lesions) was used solely for histological examination. To accomplish this, the region surrounding the injection sites was blocked and rapidly frozen in isopentane (-30°C) and stored at -80°C . Cresyl violet (FD Neurotechnologies, Baltimore, MD) staining was used to determine injection sites and neuronal viability from cryostat-generated 12- μm coronal sections surrounding the cannulae placements that were postfixed with 4% paraformaldehyde (Fisher Scientific Co., Pittsburgh, PA). In another randomly selected set of control ($n = 13$) and 6-OHDA-lesioned rats ($n = 13$), striata were freshly dissected +0.5 mm to -0.5 mm around the injection site, frozen at -80°C , and later subjected to monoamine analysis using high-performance liquid chromatography (HPLC) with electrochemical detection to determine levels of DA depletion.

HPLC Analysis. HPLC with electrochemical detection was performed on striatal tissue as previously described (Gresch and Walker, 1999). The signals produced by the oxidation of monoamines and metabolites were measured and compared with those of known concentrations of high quality standards. The final oxidation current values were adjusted to milligrams of protein amounts determined by Lowry protein assay and expressed as nanograms of monoamine per milligram of protein (mean \pm S.E.).

Data Analysis. All behavioral data were expressed as means \pm S.E. and analyzed by one-way analysis of variance (ANOVA) using Statistica software '98 (StatSoft, Tulsa, OK). When appropriate, post hoc comparisons were carried out with Fisher's least square design post hoc tests. For monoamine analysis, single-group (lesioned versus intact) differences were determined by independent two-tailed Student's *t* tests. Alpha values were set to $p < 0.05$.

Results

Representative Histology. Fig. 1 shows a schematic representation of a coronal brain section (Paxinos and Watson, 1998) located 0.48 mm anterior to bregma illustrating placements for control (clear ovals) and 6-OHDA-lesioned (shaded ovals) rats included in the study. All rats analyzed were found to have injector placements within the dorsal aspects of the striatum.

Monoamine and Metabolite Levels. As shown in Table 1, HPLC analyses revealed a significant decline in striatal

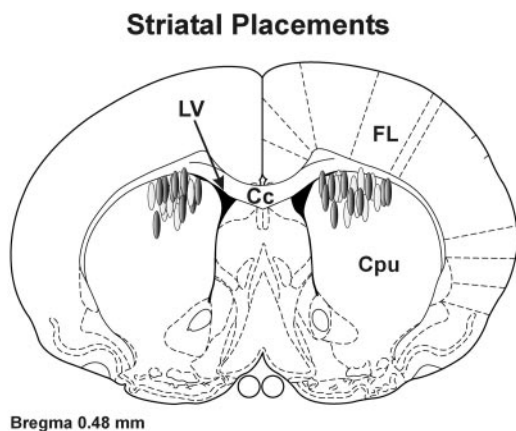


Fig. 1. Schematic representation of coronal sections of the rat brain depicting the distribution of striatal microinfusion sites. The section was taken from Paxinos and Watson (1998). Shaded and clear ovals denote placements within the striatum for selected 6-OHDA-lesioned and intact control rats, respectively. Relevant anatomical structures are: Cc, corpus callosum; Cpu, caudate putamen; FL, forelimb area of the cortex; and LV, lateral ventricle.

TABLE 1

Effect of neonatal bilateral intracerebroventricular 6-OHDA treatment on concentrations of norepinephrine (NE), DOPAC, DA, 5-HIAA, 5-HT, and their metabolite/monoamine ratios in the striatum

Values are nanograms of monoamine or metabolite per milligrams of protein or ratios of metabolite/monoamine (mean \pm S.E.) with percentage of vehicle group in parentheses.

Group	NE	DOPAC	DA	DOPAC/DA	5-HIAA	5-HT	5-HIAA/5-HT
Vehicle ($n = 13$)	0.10 \pm 0.02	10.68 \pm 0.78	48.58 \pm 4.08	0.23 \pm 0.02	1.77 \pm 0.12	1.01 \pm 0.07	1.83 \pm 0.12
6-OHDA ($n = 13$)	0.13 \pm 0.02 (126)	0.62 \pm 0.08 (5.7)*	0.93 \pm 0.15 (1.9)*	0.72 \pm 0.07 (313)*	3.23 \pm 0.50 (182)*	1.97 \pm 0.10 (195)*	1.65 \pm 0.24 (90)

*Differences between group means were determined by independent two-tailed Student's *t* tests ($p < 0.05$ compared with vehicle group).

DA (-98%) and DOPAC (-94%) levels in the 6-OHDA-injected group compared with intact control rats, whereas the DOPAC/DA ratio increased (+213%), indicating heightened DA turnover in a small percentage of surviving DA terminals. Increased 5-HT (+95%) and 5-HIAA (+82%) levels were indicative of the 5-HT hyperinnervation phenomenon, but 5-HIAA/5-HT ratios remained at control levels. Norepinephrine amounts in lesioned striata were not significantly different from controls. Such results are consistent with our previous studies (Basura and Walker, 2000, 2001; Campbell et al., 2001).

Locomotor Effects of Systemic DOI. Peripheral administration of 5-HT₂ receptor agonists has been reported to have subtle but significant effects on motor activity (Willins and Meltzer, 1997). To this point, however, it is not known whether DA depletion modifies these effects. In the present study, systemic administration of DOI produced a number of dose-dependent effects on locomotor behavior that were enhanced by 6-OHDA lesions (Fig. 2), including horizontal activity [$F_{(5,57)} = 8.91$; $p < 0.001$], total distance [$F_{(5,57)} = 8.33$; $p < 0.001$], movement bouts [$F_{(5,57)} = 11.35$; $p < 0.001$], stereotypy activity [$F_{(5,57)} = 6.43$; $p < 0.001$], and stereotypy bouts [$F_{(5,57)} = 11.01$; $p < 0.001$]. Post hoc tests on each variable demonstrated that 6-OHDA-lesioned rats receiving either 0.2 or 2.0 mg/kg DOI were more active than if they received vehicle (all $p < 0.05$). In contrast, control rats only displayed enhanced activity following 2.0 mg/kg DOI on the variables movement bouts and stereotypy bouts (both $p < 0.05$). Furthermore, 6-OHDA-lesioned rats were found to be more active than controls on all variables following 2.0 mg/kg DOI (all $p < 0.05$).

Effects of Intrastratial 5-HT₂ Agonism on Locomotor Behavior. To determine whether the effects of systemic DOI treatment may have been mediated by neurons of the striatum, we infused DOI into the dorsal striatum of control and 6-OHDA-lesioned rats (Fig. 3). Initial analyses across the entire 60-min postinfusion period yielded no treatment effects (data not shown); however, it was noted that during the second 30 min of measurement, DOI-treated rats appeared more active. As such, data reported here represent means for the second 30 min of treatment. ANOVAs on treatment effects revealed significant effects on horizontal activity [$F_{(5,49)} = 2.68$; $p < 0.05$], total distance [$F_{(5,49)} = 2.80$; $p < 0.05$], stereotypy activity [$F_{(5,49)} = 2.68$; $p < 0.05$], and stereotypy bouts [$F_{(5,49)} = 3.16$; $p < 0.05$] but not movement bouts. Post hoc testing demonstrated that 6-OHDA rats infused with either 1.0 or 10 μ g DOI were more active than if they were infused with vehicle (all $p < 0.05$). Importantly, 6-OHDA-lesioned rats were more active than controls following 1.0 (all $p < 0.05$) or 10 (all $p < 0.05$) μ g of DOI, with the exception of stereotypy activity, where no differences were found with the 10- μ g dose of DOI.

Effects of 5-HT₂ Receptor Antagonists on DOI-Stimulated Behavior. Because some of the locomotor effects of DOI originated from the striatum, the receptor specificity of these effects were explored. The lower dose (1.0 μ g) of DOI was coinfused into the striatum with ketanserin (greater selectivity for 5-HT_{2A} versus 5-HT_{2C}), RS102221 (greater selectivity for 5-HT_{2C} versus 5-HT_{2A}), or M100907 (greater selectivity for 5-HT_{2A} versus 5-HT_{2C}) of intact and 6-OHDA-lesioned rats (Fig. 4). The effects of each antagonist alone were also measured. Data reported here also represent means for the second

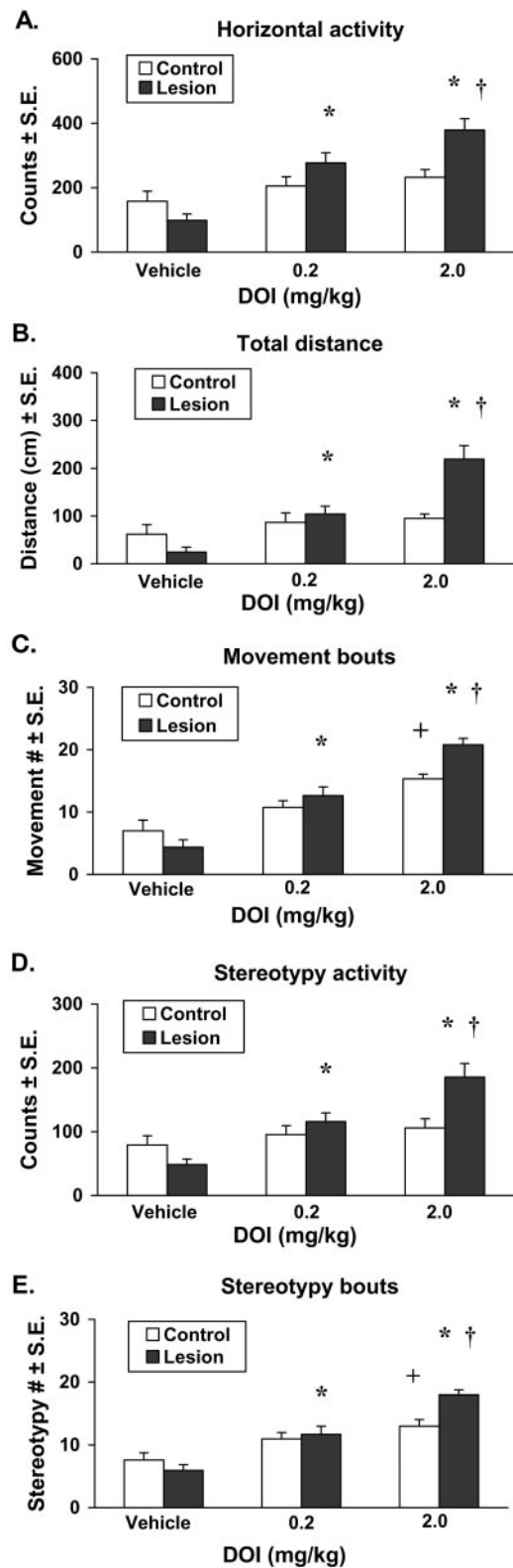


Fig. 2. Motor effects of systemic DOI (0, 0.2, and 2.0 mg/kg i.p.) in intact control and 6-OHDA-lesioned rats. Graphs denote averages of twenty 3-min measurement periods for horizontal activity \pm S.E. (A), total distance \pm S.E. (B), movement bouts \pm S.E. (C), stereotypy activity \pm S.E. (D), and stereotypy bouts \pm S.E. (E). Statistical differences were determined by one-way ANOVAs analyzing treatment effects. Post hoc comparisons on a limited number of variables are denoted by the following symbols: *, $p < 0.05$ versus vehicle-lesion; †, $p < 0.05$ versus vehicle-control; ‡, $p < 0.05$ versus 0.2-lesion and 2.0-control.

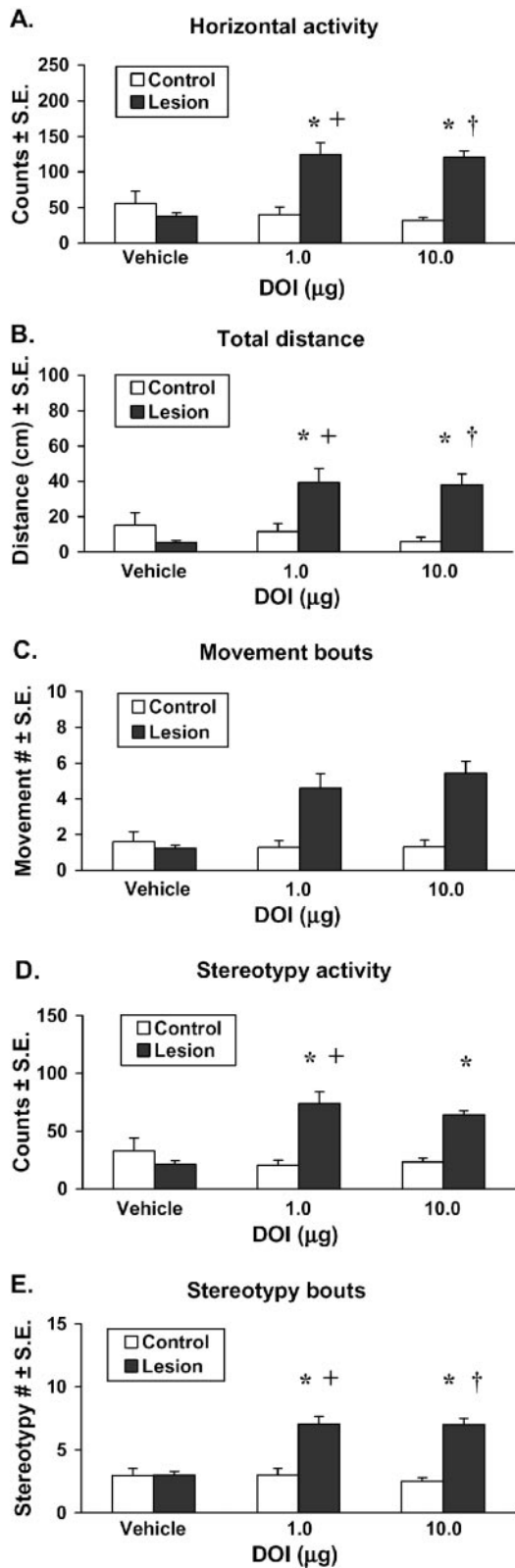


Fig. 3. Motor effects of local infusion of DOI (0, 1.0, and 10 µg/side) into dorsal striatum of intact control and 6-OHDA-lesioned rats. Graphs denote averages of ten 3-min measurement periods in the second 30 min of testing for horizontal activity ± S.E. (A), total distance ± S.E. (B), movement bouts ± S.E. (C), stereotypy activity ± S.E. (D), and stereotypy bouts ± S.E. (E). Statistical differences were determined by one-way ANOVAs analyzing treatment effects. Post hoc comparisons on a limited number of variables are denoted by the following symbols: *, $p < 0.05$ versus vehicle-lesion; +, $p < 0.05$ versus 1.0-control; and †, $p < 0.05$ versus 10-control.

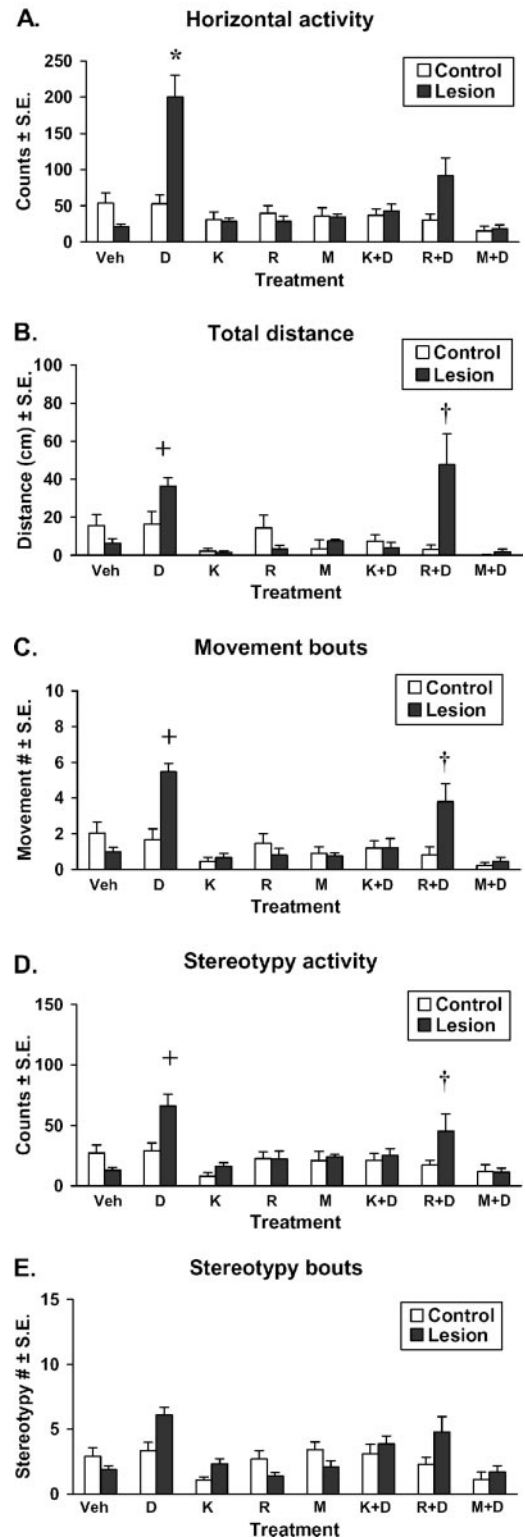


Fig. 4. Motor effects of vehicle (Veh), DOI (D; 1.0 µg), ketanserin (K; 1.0 µg), RS102221 (R; 1.0 µg), M100907 (M; 1.0 µg), or DOI cofused with each antagonist (K+D, R+D, and M+D; 1.0 µg + 1.0 µg) into the dorsal striatum of intact control and 6-OHDA-lesioned rats. Graphs denote averages of ten 3-min measurement periods in the second 30 min of testing for horizontal activity ± S.E. (A), total distance ± S.E. (B), movement bouts ± S.E. (C), stereotypy activity ± S.E. (D), and stereotypy bouts ± S.E. (E). Statistical differences were determined by one-way ANOVAs analyzing dose effects. Post hoc comparisons on a limited number of variables are denoted by the following symbols: *, $p < 0.05$ versus all; +, $p < 0.05$ versus all Lesion conditions except R + D; †, $p < 0.05$ versus vehicle-lesion but not DOI-lesion.

30-min period of treatment. ANOVAs revealed significant treatment effects on horizontal activity [$F_{(15,125)} = 3.29; p < 0.001$], total distance [$F_{(5,125)} = 1.76; p < 0.05$], movement bouts [$F_{(15,125)} = 2.54; p < 0.01$], and stereotypy activity [$F_{(15,125)} = 1.78; p < 0.05$] but not stereotypy bouts. Although motor activity tended to be lower in lesioned rats treated with vehicle versus intact controls, these differences were not statistically significant. Post hoc testing reinforced our previous findings by showing that 6-OHDA rats infused with 1.0 μg of DOI were more active than if they were infused with vehicle or compared with control rats receiving 1.0 μg of DOI (all $p < 0.05$). Although alone no antagonist influenced activity in either lesion or control groups, ketanserin and M100907 significantly attenuated DOI-induced activity on each variable (all $p < 0.05$). In contrast, RS102221 did not reduce DOI-induced activity in 6-OHDA-lesioned rats on measures of total distance, movement bouts, or stereotypy activity.

Discussion

The present study showed that systemic 5-HT₂ agonist treatment more potently stimulates motor activity within DA-depleted rats compared with intact controls. When DOI was confined to the striatum via local infusion, hyperactivity was again observed in DA-depleted rats but not in intact controls. The locomotor effects of intrastriatal DOI were completely suppressed by confusion of the nonselective 5-HT₂ receptor antagonist ketanserin as well as the selective 5-HT_{2A} receptor antagonist M100907 but not by the purported 5-HT_{2C} antagonist RS102221. These results suggest that striatal 5-HT_{2A} receptors underlie increased motor behaviors in the DA-depleted rat induced by local striatal 5-HT₂ receptor stimulation. Coupled with prior gene expression analysis, this study implicates the direct striatal output pathway as the circuit that underlies the heightened locomotor behavior.

In the intact rat, a well characterized motoric behavior induced by systemic DOI treatment is the head-twitch response (Darmani et al., 1992), now known to be mediated by 5-HT_{2A} receptors of the medial prefrontal cortex (Willins and Meltzer, 1997). In contrast, systemic DOI alone seems to produce only modest effects on locomotor behavior (Koskinen et al., 2000). Such results are consistent with observations of the present study showing that only movement bouts and stereotypic activity were increased in intact animals treated with the higher systemic DOI dose (2.0 mg/kg). 5-HT_{2A} and 5-HT_{2C} receptor-mediated signaling mechanisms within the basal ganglia have been suggested to provide significant regulatory control over motor activity when combined with other compounds such as DA stimulants (O'Neill et al., 1999; McMahon et al., 2001; Filip and Cunningham, 2002). In addition, previous studies have implicated the 5-HT₂ receptor system in spontaneous hyperactivity induced by neonatal DA depletion (Luthman et al., 1991). This latter observation is in agreement with the findings of the present study that lower systemic DOI doses were potent inducers of ambulation and stereotypic movements in DA-depleted animals.

It is interesting that neither low nor high doses of DOI induced a behavioral change in the intact animal following intrastriatal infusion in the present study. This result is not surprising given the fact that the rostral striatum exhibits weak DOI binding under normal conditions (McKenna et al.,

1989; Appel et al., 1990). In agreement, direct pathway expression of PPT mRNA was unaffected in the rostral striatum following systemic DOI treatment but was increased in the caudal striatum where 5-HT_{2A} receptors are more heavily expressed (Walker et al., 1996; Gresch and Walker, 1999; Basura and Walker, 2001). Thus, although DOI may not be a strong regulator of locomotor behavior controlled by direct pathway neurons of the intact rostral striatum, it may have a greater influence if placed into the caudal striatum where PPT mRNA is more readily induced by this agonist. In contrast to the results of intrastriatal DOI infusions in the intact animal, the present study found that both low and high DOI doses induced locomotor and stereotypic behavior when infused into the rostral striatum of DA-depleted rats. This result is consistent with our previous observations that DOI provided a more effective regulation of striatal PPT mRNA expression in DA-depleted animals (Gresch and Walker, 1999; Basura and Walker, 2001). Increased 5-HT_{2A} receptor biosynthesis in the DA-depleted rostral striatum, as indicated by an increased level of [¹²⁵I]DOI binding and 5-HT_{2A} receptor mRNA expression (Radja et al., 1993; Laprade et al., 1996; Basura and Walker, 1999), may provide the means for the enhanced actions of DOI within the DA-depleted striatum. Together, these results strengthen our hypothesis that motor behavior induced in DA-depleted rats following local striatal 5-HT₂ receptor stimulation involves the activation of the direct striatal output pathway.

Antagonists were included in the design of this study that have nanomolar binding affinities for 5-HT_{2A} versus 5-HT_{2C} receptors with 100-fold selectivity in discriminating each subtype (Johnson et al., 1996; Kehne et al., 1996; Bonhaus et al., 1997). Although the present study did not determine whether antagonist concentrations within the injection site were in the range that would ensure selectivity, results showing the differential blocking effects of each antagonist help discern which receptor is likely involved in DOI-induced motor activities. Such data are important because although (\pm)-DOI binds selectively to 5-HT₂ receptors, this agonist is unable to discriminate between 5-HT_{2A} and 5-HT_{2C} subtypes (McKenna et al., 1989; Appel et al., 1990). Though 5-HT_{2C} receptors do not appear to be increased within the striatum in response to DA depletion (Basura and Walker, 1999), they are thought to provide significant regulation of signal transduction when stimulated (Wolf and Schutz, 1997). Interestingly, 5-HT_{2C} antagonists administered locally into the substantia nigra have been shown to potentiate movements induced by DA receptor agonists in the DA-depleted rat (Fox et al., 1998). However, intrastriatal infusion of the selective 5-HT_{2C} antagonist RS102221 (Bonhaus et al., 1997) had no effect on motor activity in the present study, whereas ketanserin and M100907 both suppressed DOI-induced movements in DA-depleted rats. Ketanserin was originally developed as a selective 5-HT₂ receptor antagonist, but it is also known to possess significant histamine and α 1-adrenergic binding properties (Leysen et al., 1982). However, ketanserin is still useful to help discriminate between 5-HT₂ subtypes since it demonstrates superior antagonist properties at 5-HT_{2A} versus 5-HT_{2C} receptors (Jerman et al., 2001). The more recent development of M100907 (formerly MDL 100,907) has aided in further delineation of 5-HT₂ receptor subtypes since it binds the 5-HT_{2A} receptor with subnanomolar affinity in contrast to moderate to low (>100 fold

separation) affinity at the 5-HT_{2C} and α_1 -adrenergic sites and >500-fold separation from D₂ receptor binding (Johnson et al., 1996; Kehne et al., 1996). Thus, our observations that two 5-HT_{2A} receptor antagonists showed blocking effects whereas a 5-HT_{2C} receptor antagonist was without effect supports our hypothesis that a strengthened 5-HT_{2A} receptor system underlies the ability of intrastriatal DOI to produce movement in the DA-depleted rat. In accordance with these results, previous studies demonstrated that ketanserin suppressed the effects of systemic DOI treatment on striatal PPT mRNA induction in the DA-depleted rat (Basura and Walker, 2001).

Finally, although the present findings were uncovered in rodents, the results obtained may have relevance to human disorders of movement. Since the triggering mechanism for striatal 5-HT_{2A} receptor plasticity in rodents is DA depletion and not 5-HT sprouting (Laprade et al., 1996; Basura and Walker, 1999), enhanced 5-HT_{2A} receptor signaling may influence striatal function in adult disorders that deplete DA but do not necessarily induce the 5-HT hyperinnervation phenomenon. It may be significant that 5-HT_{2A} receptors are intensely expressed within striosomes of the human caudate nucleus and putamen (Waeber and Palacios, 1994; Lopez-Gimenez et al., 1999) and thus may play a prominent role in the regulation of striatal function. Such approximation may be relevant when considering the possibility that oversensitive 5-HT_{2A} receptors may contribute to unwanted movements, such as dyskinesias that develop in response to levodopa treatment in Parkinson's disease or excessive behaviors that accompany attention-deficit hyperactivity disorder. Indeed, recent evidence obtained from DA-depleted rodents suggests that striatal 5-HT_{2A} receptor plasticity contributes to abnormal movements in response to intrastriatal D₁ receptor stimulation (Bishop and Walker, 2003; Bishop et al., 2003). Therefore, although the phenomenon of 5-HT_{2A} receptor plasticity has been known for some years in rodents, the most interesting findings relevant to human movement disorders are yet to be revealed.

In summary, this study demonstrated that hyperlocomotive behaviors in rodents with near-complete depletion of DA were elicited by systemic or local intrastriatal infusion of the 5-HT₂ receptor agonist DOI. The behavioral effects of intrastriatal DOI infusion in DA-depleted rats were completely suppressed by coinfusion of the 5-HT_{2A} receptor antagonists M100907 or ketanserin but not the 5-HT_{2C} receptor antagonist RS102221. Since the direct striatal output pathway has been shown to be sensitive to 5-HT_{2A} receptor stimulation under conditions of DA depletion, future studies will examine the involvement of this pathway in hyperkinetic behaviors that may be relevant to human movement disorders currently treated with DA stimulants.

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References

- Appel NM, Mitchell WM, Garlick RK, Glennon RA, Teitler M, and De Souza EB (1990) Autoradiographic characterization of (\pm)-1-(2,5-dimethoxy-4-[¹²⁵I]iodophenyl)-2-aminopropane ([¹²⁵I]DOI) binding to 5-HT₂ and 5-HT_{1C} receptors in rat brain. *J Pharmacol Exp Ther* **255**:843–857.
- Basura GJ and Walker PD (1999) Serotonin_{2A} receptor mRNA levels in the neonatal dopamine-depleted rat striatum remain upregulated following suppression of serotonin hyperinnervation. *Dev Brain Res* **116**:111–117.
- Basura GJ and Walker PD (2000) Stimulated serotonin release from hyperinnervated terminals subsequent to neonatal dopamine depletion regulates striatal tachykinin, but not enkephalin gene expression. *Mol Brain Res* **81**:80–91.
- Basura GJ and Walker PD (2001) Serotonin_{2A} receptor regulation of striatal neuropeptide gene expression is selective for tachykinin, but not enkephalin neurons following dopamine depletion. *Mol Brain Res* **92**:66–77.
- Berger TW, Kaul S, Stricker EM, and Zigmond MJ (1985) Hyperinnervation of the striatum by dorsal raphe afferents after dopamine-depleting brain lesions in neonatal rats. *Brain Res* **336**:354–358.
- Bishop C, Kamdar DP, and Walker PD (2003) Intrastriatal serotonin 5-HT₂ receptors mediate dopamine D₁-induced hyperlocomotion in 6-hydroxydopamine-lesioned rats. *Synapse* **50**:164–170.
- Bishop C and Walker PD (2003) Combined intrastriatal dopamine D₁ and serotonin 5-HT₂ receptor stimulation reveals a mechanism for hyperlocomotion in 6-hydroxydopamine-lesioned rats. *Neuroscience* **121**:649–657.
- Bonhaus DW, Weinhardt KK, Taylor M, Desouza A, McNeely PM, Szczepanski K, Fontana DJ, Trinh X, Rocha CL, Dawson MW, et al. (1997) RS-102221: a novel high affinity and selective, 5-HT_{2C} receptor antagonist. *Neuropharmacology* **36**:621–629.
- Breese GR, Baumeister AA, McCown TJ, Emerick SG, Frye GD, Crotty K, and Mueller RA (1984) Behavioral differences between neonatal and adult 6-hydroxydopamine-treated rats to dopamine agonists: relevance to neurological symptoms in clinical syndromes with reduced brain dopamine. *J Pharmacol Exp Ther* **231**:343–354.
- Campbell BM, Gresch PJ, and Walker PD (2001) Neonatal dopamine depletion reveals a synergistic mechanism of mRNA regulation that is mediated by dopamine(D₁) and serotonin(2) receptors and is targeted to tachykinin neurons of the dorsomedial striatum. *Neuroscience* **105**:671–680.
- Darmani NA, Martin BR, and Glennon RA (1992) Behavioral evidence for differential adaptation of the serotonergic system after acute and chronic treatment with (\pm)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) or ketanserin. *J Pharmacol Exp Ther* **262**:692–698.
- Descarries L, Soghomonian JJ, Garcia S, Doucet G, and Bruno JP (1992) Ultrastructural analysis of the serotonin hyperinnervation in adult rat neostriatum following neonatal dopamine denervation with 6-hydroxydopamine. *Brain Res* **569**:1–13.
- Filip M and Cunningham KA (2002) Serotonin 5-HT_{2C} receptors in nucleus accumbens regulate expression of the hyperlocomotive and discriminative stimulus effects of cocaine. *Pharmacol Biochem Behav* **71**:745–756.
- Fox SH, Moser B, and Brotchie JM (1998) Behavioral effects of 5-HT_{2C} receptor antagonism in the substantia nigra zona reticulata of the 6-hydroxydopamine-lesioned rat model of Parkinson's disease. *Exp Neurol* **151**:35–49.
- Gresch PJ and Walker PD (1999) Serotonin-2 receptor stimulation normalizes striatal prepro-tachykinin mRNA in an animal model of Parkinson's disease. *Neuroscience* **93**:831–841.
- Jackson D and Abercrombie ED (1992) In vivo neurochemical evaluation of striatal serotonergic hyperinnervation in rats depleted of dopamine at infancy. *J Neurochem* **58**:890–897.
- Jerman JC, Brough SJ, Gager T, Wood M, Coldwell MC, Smart D, and Middlemiss DN (2001) Pharmacological characterization of human 5-HT₂ receptor subtypes. *Eur J Pharmacol* **414**:23–30.
- Johnson MP, Siegel BW, and Carr AA (1996) [³H]MDL 100,907: a novel selective 5-HT_{2A} receptor ligand. *Naunyn-Schmiedeberg's Arch Pharmacol* **354**:205–209.
- Kehne JH, Baron BM, Carr AA, Chaney SF, Elands J, Feldman DJ, Frank RA, Van Giersbergen PLM, McCloskey TC, Johnson MP, et al. (1996) Preclinical characterization of the potential of the putative atypical antipsychotic MDL 100,907 as a potent 5-HT_{2A} antagonist with a favorable CNS safety profile. *J Pharmacol Exp Ther* **277**:968–981.
- Koskinen T, Ruotsalainen S, and Sirvio J (2000) The 5-HT₂ receptor activation enhances impulsive responding without increasing motor activity in rats. *Pharmacol Biochem Behav* **66**:729–738.
- Laprade N, Radja F, Reader TA, and Soghomonian JJ (1996) Dopamine receptor agonists regulate levels of the serotonin 5-HT_{2A} receptor and its mRNA in a subpopulation of rat striatal neurons. *J Neurosci* **16**:3727–3736.
- Leyssen JE, Niemegeers CJE, Van Nueten JM, and Laduron PM (1982) [³H]Ketanserin (R 41 468), a selective [³H]-ligand for serotonin₂ receptor binding sites: binding properties, brain distribution and functional role. *Mol Pharmacol* **21**:301–314.
- Lopez-Gimenez JF, Mengod G, Palacios JM, and Vilaro MT (1999) Human striosomes are enriched in 5-HT_{2A} receptors: autoradiographical visualization with [³H]MDL100,907, [¹²⁵I](\pm)DOI and [³H]ketanserin. *Eur J Neurosci* **11**:3761–3765.
- Luthman J, Bolioli B, Tsutsumi T, Vershofstad A, and Jonsson G (1987) Sprouting of striatal serotonin nerve terminals following selective lesions of nigro-striatal dopamine neurons in neonatal rat. *Brain Res Bull* **19**:269–274.
- Luthman J, Fredriksson A, Plaznik A, and Archer T (1991) Ketanserin and mianserin treatment reverses hyperactivity in neonatally dopamine-lesioned rats. *J Psychopharmacol* **5**:418–425.
- McMahon LR, Filip M, and Cunningham KA (2001) Differential regulation of the mesoaccumbens circuit by serotonin 5-hydroxytryptamine (5-HT)_{2A} and 5-HT_{2C} receptors. *J Neurosci* **21**:7781–7787.
- McKenna DJ, Nazarali AJ, Hoffman AJ, Nichols DE, Mathis CA, and Saavedra JM (1989) Common receptors for hallucinogens in rat brain: a comparative autoradiographic study using [¹²⁵I]-LSD and [¹²⁵I]-DOI, a new psychotomimetic radioligand. *Brain Res* **476**:45–56.
- Molina-Holgado E, Dewar KM, Descarries L, and Reader TA (1994) Altered dopamine and serotonin metabolism in the dopamine-denervated and serotonin-hyperinnervated neostriatum of adult rat after neonatal 6-hydroxydopamine. *J Pharmacol Exp Ther* **270**:713–721.
- Mrimi A, Soucy J-P, Lafaille F, Lemoine P, and Descarries L (1995) Quantification of the serotonin hyperinnervation in adult rat neostriatum after neonatal 6-hydroxydopamine lesion of nigral dopamine neurons. *Brain Res* **669**:303–308.
- O'Neill MF, Heron-Maxwell CL, and Shaw G (1999) 5-HT₂ receptor antagonism reduces hyperactivity induced by amphetamine, cocaine and MK-801 but not D1 agonist C-APB. *Pharmacol Biochem Behav* **63**:237–243.

- Paxinos G and Watson C (1998) *The Rat Brain in Stereotaxic Coordinates*, 4th ed, Academic Press, San Diego, CA.
- Radja F, Descarries L, Dewar KM, and Reader TA (1993) Serotonin 5-HT₁ and 5-HT₂ receptors in adult rat brain after neonatal destruction of nigrostriatal dopamine neurons: a quantitative autoradiographic study. *Brain Res* **606**:273–285.
- Snyder AM, Zigmond MJ, and Lund RD (1986) Sprouting of serotonergic afferents into striatum after dopamine-depleting lesions in infant rats: a retrograde transport and immunocytochemical study. *J Comp Neurol* **245**:274–281.
- Stachowiak MK, Bruno JP, Snyder AM, Stricker EM, and Zigmond MJ (1984) Apparent sprouting of striatal serotonergic terminals after dopamine-depleting brain lesions in neonatal rats. *Brain Res* **291**:164–167.
- Towle AC, Criswell HE, Maynard EH, Lauder JM, Loh TH, Mueller RA, and Breese G (1989) Serotonergic innervation of the rat caudate following a neonatal 6-hydroxydopamine lesion: an anatomical, biochemical and pharmacological study. *Pharmacol Biochem Behav* **34**:367–374.
- Ullrich T and Rice KC (2000) A practical synthesis of the serotonin 5-HT_{2A} receptor antagonist MDL 100907, its enantiomer and their 3-phenolic derivatives as precursors for [¹¹C]labeled PET ligands. *Bioorganic Med Chem* **8**:2427–2432.
- Waeber C and Palacios JM (1994) Binding sites for 5-hydroxytryptamine-2 receptor agonists are predominantly located in striosomes in the human basal ganglia. *Mol Brain Res* **24**:199–209.
- Walker PD, Capodilupo JG, Wolf WA, and Carlock LR (1996) Preprotachykinin and preproenkephalin mRNA expression within striatal subregions in response to altered serotonin transmission. *Brain Res* **732**:25–35.
- Willins DL and Meltzer HY (1997) Direct injection of 5-HT_{2A} receptor agonists into the medial prefrontal cortex produces a head-twitch response in rats. *J Pharmacol Exp Ther* **282**:699–706.
- Wolf WA and Schutz LJ (1997) The serotonin 5-HT_{2C} receptor is a prominent serotonin receptor in basal ganglia: evidence from functional studies on serotonin-mediated phosphoinositide hydrolysis. *J Neurochem* **69**:1449–1458.

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