

Serotonin 5-HT_{2A} receptors underlie increased motor behaviors induced in dopamine-depleted rats by intrastriatal 5-HT_{2A/2C} agonism.

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Abbreviations: DA, 3-hydroxytyramine (dopamine); 5-HT, 5-hydroxytryptamine (serotonin); PPT, preprotachykinin; 6-OHDA, 6-hydroxydopamine; DMSO, Dimethyl sulfoxide; 5-HIAA, 5-hydroxyindole-3-acetic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; L-DOPA, L-dihydroxyphenylalanine.

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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 intrastriatal infusion. Motor behavior was quantified for 60 min after agonist injection using computerized activity monitors. Systemic DOI treatment (0.2-2.0 mg/kg ip) was more effective in inducing motor activity in the DA-depleted group as compared to intact controls. Intrastriatal DOI infusion (1.0 or 10.0 µg/side) also produced a significant rise in motor activity in the DA-depleted group during the 30-60 min period of behavioral analysis, but did not influence behavior in intact animals. The effects of intrastriatal DOI infusion were blocked by intrastriatal co-infusion of the 5-HT₂ antagonist ketanserin (1.0 µg) and the 5-HT_{2A}-preferring antagonist M100907 (1.0 µg), but not the 5-HT_{2C}-preferring antagonist RS 102221 (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 raphestriatal 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). Though 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 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 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). In order 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 co-infusion of selective antagonists M100907 (Kehne et al., 1996; Johnson et al., 1996) and RS102221 (Bonhaus et al., 1997) to discern the effects of local striatal 5-HT_{2A} vs 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.

Methods

Animals. Male Sprague-Dawley rats pups (Charles River Laboratories, 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” (Institute of Laboratory Animal Resources, National Academy Press 1996; NIH publication number 85-23, revised 1996). Rat pups arrived on postnatal day 2 and were initially group-housed (n=12/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 0700 h). 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-hydroxydopamine (6-OHDA) lesions and cannulae implantation surgeries. Bilateral intracerebroventricular (icv) 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, St. Louis, MO) or its vehicle solution of saline containing 0.1% ascorbic acid (Sigma) at a rate of 5 µl over 30 sec into each ventricle (AP 0.0 mm, ML ±1.0 mm, DV -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) 30 min prior to surgery to protect norepinephrine neurons. Sixty days following icv lesions, rats were anesthetized with an intraperitoneal (ip) injection of ketamine (90 mg/kg; Lloyd Laboratories,

Shenandoah, IO) 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 AP +0.4 mm, ML \pm 2.9 mm and DV - 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 ((±)-1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane hydrochloride; Sigma); ketanserin (3-[2-[4-(4-Fluorbenzoyl)-1-piperidinyl]ethyl]-2,4(1H,3H)-quinazolinedione tartrate; Sigma); M100907 ((R)-(+)- α -(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl) ethyl]-4-piperidinemethanol; synthesized as described in Ullrich and Rice, 2000); RS102221 (8-[5-(2,4-dimethoxy-5-(4-trifluoromethylsulpho-amido)phenyl)-5-oxopentyl]-1,3,8-triazaspiro[4.5]decane-2,4-dione; Tocris, 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 ip injection of saline vehicle or the 5-HT_{2A/2C} agonist DOI using 0.2 mg/kg or 2.0 mg/kg doses in a repeated-measures design with groups counter-balanced 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 μ g) at a volume of 0.8 μ l/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% DMSO in saline), 1.0 μ g DOI, 1.0 μ g of the 5-HT₂ antagonist ketanserin, 1.0 μ g of the 5-HT_{2C}-preferring antagonist RS102221, 1.0 μ g of the 5-

HT_{2A}-preferring antagonist M100907, or DOI co-infused with each of these antagonists (1.0 µg agonist + 1.0 µg antagonist) at a volume of 0.8 µl/side in a counter-balanced design. The number of animals per group ranged from 7 to 10 rats. No rat was tested more than 3 times and at least 3 days separated each testing session.

Behavioral procedure. Locomotor activity monitoring was conducted in 6 identical acrylic chambers measuring 40 cm long, 40 cm wide and 30 cm high (Accuscan Instruments, Columbus, OH). Each chamber was surrounded by a 15x15 infrared photocell array interfaced with a computer that ran the Versamax and Versadat programs (Accuscan Instruments). 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 3 times. All testing occurred between 0830-1230 h. 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, Boston, 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 sec. Immediately after the injectors were removed, the pump was re-started 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, 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 post-fixed with 4% paraformaldehyde (Fisher Scientific, Hanover Park, IL). 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 with electrochemical detection (HPLC-ED) to determine levels of DA depletion.

HPLC Analysis. HPLC-ED 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 mg protein amounts determined by Lowry protein assay and expressed as nanogram monoamine per milligram protein (mean ± S.E.).

Data Analysis. All behavioral data were expressed as means ± S.E. and analyzed by 1-way analyses of variance (ANOVAs) using Statistica software '98 (Statsoft Inc., 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 vs. intact) differences were determined by independent 2-tailed 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 (unfilled ovals) and 6-OHDA lesioned (filled 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 DA (-98%) and DOPAC (-94%) levels in the 6-OHDA-injected group as compared to intact control rats while DOPAC/DA ratio increased +313% 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 intrastriatal 5-HT₂ agonism on locomotor behavior. To determine if 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 neonate 6-OHDA-lesioned rats (Fig. 3). Initial analyses across the entire 60 min post-infusion period yielded no treatment effects (data not shown), however, it was noted that during the 2nd 30 min of measurement, DOI-treated rats appeared more active. As such, data reported here represent means for the 2nd 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 μ g (all $p < 0.05$) or 10 μ g (all $p < 0.05$) 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 co-infused into the striatum with ketanserin (greater selectivity for 5-HT_{2A} vs. 5-HT_{2C}), RS 102221 (greater selectivity for 5-HT_{2C} vs. 5-HT_{2A}), or M100907 (greater selectivity for 5-HT_{2A} vs. 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 2nd 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 vs 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 μ g DOI were more active than if they were infused with vehicle or compared to control rats receiving 1.0 μ g DOI (all $p < 0.05$). While alone no antagonist influenced activity in either lesion or control groups, ketanserin + DOI and M100907 + DOI 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 as compared to 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 co-infusion of the non-selective 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} vs 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 if 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 (±)-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 while 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} vs 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 sub-nanomolar 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

(Kehne et al., 1996; Johnson et al., 1996). Thus, our observations that two 5-HT_{2A} receptor antagonists showed blocking effects while 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 considered 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 et al., 2003; Bishop and Walker, 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 co-infusion 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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Figure Legends

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 lesion and intact control rats respectively. Relevant anatomical structures are: Cc corpus callosum, Cpu caudate putamen, FL forelimb area of the cortex, LV lateral ventricle.

Fig. 2. Motor effects of systemic DOI (0, 0.2 and 2.0 mg/kg ip) 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 1-way ANOVAs analyzing treatment effects. Post hoc comparisons on a limited number of variables are denoted by the following symbols: * p <0.05 vs. Vehicle-Lesion, + p <0.05 vs. Vehicle-Control, † p <0.05 vs. 0.2-Lesion and 2.0-Control.

Fig. 3. Motor effects of local infusion of DOI (0, 1.0 and 10 μ g/side) into dorsal striatum of intact control and 6-OHDA-lesioned rats. Graphs denote averages of ten 3 min measurement periods in the 2nd 30 min of testing 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 1-way ANOVAs analyzing treatment effects. Post hoc comparisons on a limited number of variables are denoted by the following symbols: * p <0.05 vs. Vehicle-Lesion, + p <0.05 vs. 1.0-Control, † p <0.05 vs 10-Control.

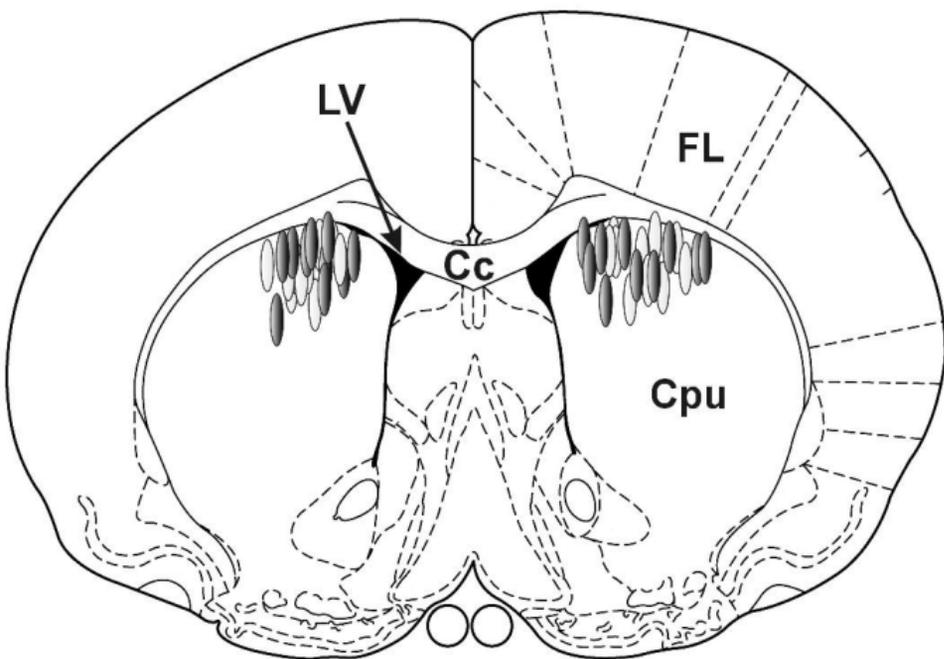
Fig. 4. Motor effects of Vehicle (Veh), DOI (D; 1.0 μ g), ketanserin (K; 1.0 μ g), RS102221 (R; 1.0 μ g), M100907 (M; 1.0 μ g), or DOI co-infused with each antagonist (K+D, R+D, and M+D; 1.0 μ g + 1.0 μ g) into the dorsal striatum of intact control and 6-OHDA-lesioned rats. Graphs denote averages of ten 3 min measurement periods in the 2nd 30 min of testing 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 1-way ANOVAs analyzing dose effects. Post hoc comparisons on a limited number of variables are denoted by the following symbols: * p <0.05 vs. all, + p <0.05 vs. Lesion conditions but R+D, † p <0.05 vs. Vehicle-Lesion and not DOI-Lesion.

Table 1. Effect of neonatal bilateral intracerebroventricular 6-hydroxydopamine (6-OHDA) treatment on concentrations of norepinephrine (NE), 3-4-dihydroxyphenylacetic acid (DOPAC), dopamine (DA), 5-hydroxyindolacetic acid (5-HIAA), serotonin (5-HT) and their metabolites /amine ratios in the striatum.

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

Values are nanogram monoamine or metabolite per milligram protein or ratios of metabolite to monoamine (mean ± S.E.) with percent of vehicle group in parentheses. Differences between group means were determined by independent 2-tailed t-tests (* $p < 0.05$ compared to vehicle group).

Striatal Placements



Bregma 0.48 mm

