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Dopamine D3 receptors are involved in amphetamine-induced contralateral rotation in 6-OHDA lesioned rats

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Abstract

The aim of the present experiment was to investigate the possibility that alterations in dopamine D3 receptors have a role in the normalization of function that occurs following a unilateral lesion of the medial forebrain bundle induced by 6-hydroxydopamine (6-OHDA). Unilateral lesions result in an enhanced rotational response to dopamine agonists that appears to be due to an increase in stimulatory D2 receptors on the lesioned side that occurs by about 1 week postlesion. The present experiment assessed the involvement of D3 receptors in rotational behavior by testing the animals at 48 h postlesion. At this time interval, D2 receptors have not become up-regulated. In contrast, D3 receptors have been shown to be down-regulated. Rats with $\geq$98% dopamine depletion induced by 6-OHDA exhibited mostly ipsilateral rotation in response to an injection of amphetamine. This rotation was not affected by pretreatment with the D3 antagonist U-99194A. Rats with 80–97% dopamine depletion exhibited mostly contralateral rotation in response to amphetamine and this rotation was blocked by pretreatment with U-99194A. In addition, a decrease in D3 receptor binding was observed by 48 h postlesion. These results support the hypothesis that the decrease in D3 receptors seen following denervation is involved in the compensatory response of the system. This may have important clinical relevance in the treatment of disorders such as Parkinson's disease and drug abuse.

Keywords: Compensatory response; Unilateral lesion; Receptor binding; D3 antagonist

1. Introduction

Dopamine receptor subtypes have been classified into two major families based on molecular, biochemical and pharmacological similarities. The D1 receptor family includes the D1 and D5 subtypes, whereas the D2 receptor family includes the D2, D3 and D4 subtypes (Missale et al., 1998; Sibley and Monsma, 1992). The recently cloned D3 receptor is thought to exist both pre- and postsynaptically (Sokoloff et al., 1990) and appears to be most abundant in the terminal regions of the mesolimbic dopamine system (Bancroft et al., 1998). Recent evidence suggests that within the D2 family, dopamine D3 receptors may be functionally distinct from other receptors in the D2 family. For example, D3 receptors inhibit behavioral activity, whereas D2 receptors activate these same systems (Heijtz et al., 2000; Menalled et al., 1999; Piercey et al., 1995; Sautel et al., 1995; Waters et al., 1993).

The neurotoxin 6-hydroxydopamine (6-OHDA) has been widely used to study dopamine function. When animals are given a unilateral 6-OHDA lesion to either the nigrostriatal or the mesolimbic dopamine pathway, they display an enhanced rotational response to dopamine agonists. The direction of the rotational behavior is dependent on the type of agonist used. Treatment with a direct-acting agonist (e.g., apomorphine) results in rotation away from the side of the brain that is lesioned. Treatment with an indirect agonist (e.g., amphetamine) results in rotation toward the lesion side (Ungerstedt, 1971).

The mechanism for this lesion-induced supersensitivity to dopamine agonists appears to be mediated by both pre- and postsynaptic compensatory mechanisms. Presynaptic changes include increases in dopamine synthesis (Hefti et al., 1985; Zigmond et al., 1984) and release (Hefti et al., 1985; Robinson and Whishaw, 1988), and decreases in

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dopamine reuptake and clearance (Cadet and Zhu, 1992; Castañeda et al., 1990). When destruction of dopamine containing neurons is extensive (>90%), postsynaptic changes also occur. Several studies have found an increase in D2 receptor binding in 6-OHDA lesioned rats without changes in receptor affinity (e.g., Creese et al., 1977; Graham et al., 1990; Narang and Wamsley, 1995). In contrast, D3 receptor binding appears to decrease after unilateral 6-OHDA lesions of the nigrostriatal and mesolimbic pathways (Bordet et al., 1997; Lévesque et al., 1995). It has been suggested that the increase in D2 receptor density, rather than the decrease in D3 receptors, is responsible for the supersensitized locomotor response to dopamine agonists in lesioned rats. However, since D3 receptors may have an inhibitory effect on activity (Heijtz et al., 2000; Menalled et al., 1999; Piercey et al., 1995; Sautel et al., 1995; Waters et al., 1993), it remains possible that the down-regulation of D3 receptors found in 6-OHDA lesioned animals contributes to the increase in sensitivity to dopamine agonists. That is, with down-regulation, the inhibitory influence of D3 receptor activation may be decreased. This would then enhance the ability of the stimulatory D2 receptors to increase activity.

In the traditional rotational behavior model (Ungerstedt, 1971), the psychostimulant amphetamine induces ipsilateral rotation in animals with unilateral 6-OHDA lesions. However, during the first few days postlesion, seemingly paradoxical amphetamine-induced contralateral rotation is observed (Carey, 1992; Labandeira-Garcia et al., 1996; Robinson et al., 1994; Ungerstedt, 1971). This contralateral rotation behavior in response to amphetamine is seen within 24 h postlesion; however, by about Day 4 postlesion, the rotations convert to the ipsilateral direction (Schwarting and Huston, 1996). Rotation away from the lesioned side of the brain (i.e., contralaterally), indicates that there is more amphetamine-induced dopamine stimulation on the lesioned side of the brain than on the non-lesioned side.

A possible explanation for the transient period of contralateral rotation lies in the mechanism by which amphetamine has its effect on the dopamine system. Amphetamine increases the release of dopamine from the nerve terminal in a manner that is insensitive to depletion of endogenous stores by reserpine (Langer and Arbilla, 1984). This indicates that amphetamine releases newly synthesized dopamine that is located outside the synaptic vesicles. Soon after denervation of dopamine terminals by 6-OHDA, synthesis of dopamine is increased in the remaining terminals (Hefti et al., 1985, Zigmond et al., 1984). This increase in synthesis may result in a larger available pool of amphetamine-releasable dopamine on the denervated side relative to the intact side. This would result in more stimulation on the lesioned side and thus, contralateral rotation would occur. In addition, other presynaptic compensatory mechanisms (i.e., decreases in reuptake and clearance) would further enhance the effect of the dopamine released by amphetamine. As recovery progresses (4–8 days postlesion), amphetamine may induce ipsilateral rotation because of continued degeneration of dopamine terminals on the lesioned side. This results in less release of dopamine on the denervated side relative to the intact side.

In addition to the presynaptic mechanisms for amphetamine-induced contralateral rotation during the first few days postlesion, it has also been suggested that the contralateral rotation is due to supersensitivity of postsynaptic D2 and possibly D1 receptors (Robinson et al., 1994). However, an increase in D2 receptor binding is not found until at least 4 days postlesion (Neve et al., 1982, Narang and Wamsley, 1995). Further, no changes in D2 binding are found 24–48 h following surgery (Staunton et al., 1981), while amphetamine-induced contralateral rotation is seen within 24–48 h postlesion (Labandeira-Garcia et al., 1996; Schwarting and Huston, 1996). In contrast to D2 receptors, D3 receptors may be down-regulated as early as 24 h postlesion (Lévesque et al., 1995). Thus, it is possible that the decrease in D3 receptors may have a role in the contralateral rotation observed in response to amphetamine.

The purpose of the present experiment was to examine the potential role of D3 receptors in the contralateral rotational response to amphetamine. When amphetamine is administered 48 h after treatment with 6-OHDA, the terminals on both sides of the brain will release dopamine. Because of the presynaptic compensatory mechanisms (described above), the spared terminals on the lesioned side may have an enhanced ability to release dopamine in response to amphetamine, resulting in more stimulation of receptors on the lesioned side. This higher stimulation on the lesioned side is the presumed mechanism to explain amphetamine-induced contralateral rotation at this postlesion time interval (Robinson et al., 1994; Ungerstedt, 1971). At this time, the inhibitory effect of D3 receptor stimulation should also be decreased on the lesioned side due to receptor down-regulation (Lévesque et al., 1995). If the loss of D3 receptors on the lesioned side has a role in contralateral rotation following amphetamine, then blockade of D3 receptors with the D3 receptor antagonist U-99194A (Kling-Petersen et al., 1995; Waters et al., 1993) should diminish amphetamine-induced contralateral rotation. More specifically, since the intact side contains a normal level of inhibitory D3 receptors, whereas the D3 receptors on the lesioned side have been decreased, amphetamine should produce a greater inhibitory effect on the intact side relative to the lesioned side. This should allow more stimulation (via stimulatory D2 receptors) on the lesioned side in response to the dopamine being released by amphetamine from the presynaptic terminal. However, if D3 receptors are blocked, the inhibitory influence of D3 stimulation on both sides of the brain is removed and thus, a balance in stimulation should be restored. This would then allow D2 receptors to mediate the activity level on both sides of the brain and result in a decrease in amphetamine-induced contralateral rotation.

A single concentration assay for D3 receptor binding was performed on nucleus accumbens tissue dissected from the saline control animals. The assay was conducted in order to replicate the decrease in D3 receptor binding that has been found previously (Bordet et al., 1997; Lévesque et al., 1995).

2. Method

2.1. Subjects

Male Sprague–Dawley rats (Harlan Industries, Indianapolis, IN), weighing 200–225 g at the start of the experiment, were individually housed in plastic cages with pine chip bedding. Food and water were available continuously in the home cage. A 12-h light/dark cycle was maintained in a temperature-controlled colony room. All behavioral testing occurred during the light phase of the cycle. Animals were handled for approximately 5 min for three consecutive days prior to the start of each experiment. All animals were treated in accordance with a protocol approved by the University of Kentucky Institutional Animal Care and Use Committee.

2.2. Drugs

The dopamine D3 receptor preferring antagonist U-99194A (RBI, Natick, MA) was dissolved in 0.9% NaCl and injected subcutaneously in a volume of 1 ml/kg body weight. Pargyline, desipramine (RBI) and D-amphetamine sulfate (Sigma, St. Louis, MO) were dissolved in 0.9% NaCl and injected intraperitoneally in a volume of 1 ml/kg body weight. Ketamine (100 mg/kg: Fort Dodge Laboratories, Fort Dodge, IA) and diazepam (5 mg/kg: Steris Laboratories, Phoenix, AZ) were obtained premixed and injected intraperitoneally in a volume of 1 ml/kg body weight.

2.3. Surgical procedure

For 6-OHDA lesions, surgical anesthesia was induced with ketamine (100 mg/kg ip) followed immediately by diazepam (5 mg/kg ip). When anesthesia was evident, the rats were injected with desipramine (25 mg/kg ip) to protect noradrenergic neurons, and pargyline (50 mg/kg ip) to inhibit endogenous monoamine oxidase (MAO) and delay catabolism of the 6-OHDA. Animals were then positioned in a Kopf stereotaxic instrument and 6-OHDA (4 μg/μl in 0.2% ascorbic acid) was injected (2 μl) into either the right or left medial forebrain bundle. The side of 6-OHDA injections (right or left) was counterbalanced across rats. The 6-OHDA was injected using a Hamilton 10-μl syringe with a blunt tip. The needle was left in place for 3 min following injection. The injection site was at the following coordinates with respect to bregma and dura: AP −2.6, ML ±2.1, DV −8.3, according to Paxinos and Watson (1986). Vehicle-injected control rats were treated the same as the lesion rats except that only the 6-OHDA vehicle (0.2% ascorbic acid) was injected at the above coordinates.

2.4. Rotational apparatus

Rotation behavior was recorded in a circular plastic tube measuring 22.5 cm in diameter and 45 cm in height. The circular tubes had white walls and black rubber floors. The rats were videotaped during each session and subsequently scored for number of contralateral and ipsilateral rotations by an observer who was unaware of group assignment. A rotation was operationally defined as the rat's head turning 360° with the animal's body as the axis of the turn. A white noise generator (70 dB ambient) was located in the test room.

2.5. Analysis of dopamine levels

Forty-eight hours following behavioral testing, the rats were killed by rapid decapitation. The brains were removed and placed on an ice-cold dissection plate. The dorsal striatum was dissected from a coronal slice that extended from approximately 0.2 to 3.0 mm anterior to bregma (Paxinos and Watson, 1986). Each brain area was weighed and put into 0.1 M $HClO_4$ and frozen at −70°C.

To assay for dopamine, the tissue was thawed and sonicated. The homogenates were centrifuged at 30,000 × *g* for 15 min at 4°C. The supernatants (50 μl) were injected into a high-pressure liquid chromatograph (HPLC) with a unijet electrode (Bioanalytical Systems, MF-2061) and a microbore Sepstik column (Bioanalytical Systems, MF-8945). The mobile phase consisted of 14.5 mM NaH2PO4, 3 mM sodium citrate, 27 μM disodium EDTA, 1.95 mM 1-decanesulfonic acid sodium salt, 10 mM NaCl, pH to 3.4 with H_3PO_4, 80 ml acetonitrile. External standards for dopamine were assayed daily with the tissue samples.

2.6. Procedure

Animals were randomly assigned to one of eight groups according to the following design:

	Vehicle	Agonist tested
Antagonist pretreatment	Saline	Amphetamine (2.5 mg/kg)
Saline	$N=11$	$N=9$
U-99194A (5 mg/kg)	$N=5$	$N=10$
U-99194A (10 mg/kg)	$N=9$	$N=8$
U-99194A (20 mg/kg)	$N=6$	$N=8$

Forty-eight hours following surgery, the rats were placed into the rotation chambers for 15 min to allow habituation to the apparatus. The rats were then injected with either saline

or U-99194A (5.0, 10.0 or 20.0 mg/kg sc) and placed in the rotation chambers. Fifteen minutes later, the animals were injected with either saline or amphetamine (2.5 mg/kg ip) and placed back into the chamber. The number of ipsilateral and contralateral rotations was then recorded for 30 min in 10-min blocks by an observer unaware of group assignment. The doses of U-99194A were selected based on previous work (Waters et al., 1993).

Immediately following behavioral testing, the saline control animals (saline–saline) were killed by rapid decapitation and their brains were removed. The dorsal striatum and nucleus accumbens were dissected as described above and frozen at −70°. The assay for dopamine was performed on the striatal tissue as described above. The D3 binding assay was then performed on the nucleus accumbens tissue according to the general methods described by Lévesque et al. (1995). Tissue samples were thawed on ice and then homogenized (Brinkman Polytron, setting 7 for 10 s) in 20 volumes of ice-cold 10 mM Tris HCl (pH 7.5) containing 1 mM EDTA. In order to perform the assay for dopamine in the nucleus accumbens tissue, 25 μl of this homogenate was removed and added to 5 μl perchloric acid (0.1 N). The assay for dopamine was then performed on this aliquot as described above. The remaining homogenate was centrifuged at 35,000 × *g* for 15 min at 4°C. The supernatant was poured off and the pellet was resuspended and centrifuged again. This washing procedure continued for a total of three washes. After the final centrifugation, the pellet was resuspended in 200 volumes incubation buffer containing 50 mM NaHepes, 1 mM EDTA, 0.005% ascorbic acid, 50 μM 8-hydroxyquinoleine, and 0.1% bovine serum albumen (pH 7.5).

The tissue suspensions were added in duplicate to polypropylene test tubes containing incubation buffer and [^{3}H]7-OH-DPAT (2 nM) in the presence and absence of 10 μM dopamine. To reach equilibrium, the incubation time was 60 min at room temperature. The binding was terminated by vacuum filtration (Whatman GF/B glass fiber filters) and unbound [^{3}H]7-OH-DPAT was washed twice from the filters by 3–4 ml ice cold buffer (50 mM Tris HCl, pH 7.5, 120 mM NaCl). The filters were presoaked in 0.1% PEI. After filtration, the filters were placed in glass vials with 8 ml scintillation fluid (Scintiverse, Sigma) and radioactivity was measured using liquid scintillation spectrometry (Packard 3380 with approximately 38% counting efficiency).

Specific binding was defined as the difference in bound radioactivity obtained in the presence and absence of 10 μM dopamine.

2.7. Data analysis

Dopamine levels (nanograms dopamine per milligram wet weight tissue) on the lesioned striatum were compared to the dopamine levels on the intact striatum and a percent depletion was calculated. Only rats with at least 80% depletion were used in the data analysis.

A mixed-factor ANOVA was performed on the behavioral data. Pretreatment with U-99194A (0.0, 5.0, 10.0 or 20.0 mg/kg sc) and treatment with either saline or amphetamine were the between groups measures. Time blocks (0–10, 10–20, and 20–30 min) was the repeated measure.

Following an initial examination of the data, it was evident that the animals were responding differently to amphetamine in terms of contralateral and ipsilateral rotation. It was hypothesized that this may be due to differences in 6-OHDA-induced dopamine depletion levels. Extensive dopamine depletion may result in the inability to compensate for the lesion (Castañeda et al., 1990). Thus, amphetamine-stimulated ipsilateral rotation should be correlated with depletion levels. A Pearson correlational analysis was used to examine the relationship between percent depletion of dopamine in the striatum and number of ipsilateral rotations in amphetamine treated animals. A correlational analysis was also performed relating percent depletion of dopamine in the striatum and contralateral rotation. A median split analysis was then performed on the rats based on percent depletion of dopamine in the striatum. Separate mixed-factor ANOVAs were then performed on the data for the rats falling at or above the median and those falling below the median. Treatment with either saline or amphetamine was a between-groups measure. Time blocks (0–10, 10–20 and 20–30 min) was a repeated measure. In the case of a significant interaction, a Newman Keuls analysis was used to test for differences between groups.

For the binding assay, a *t* test was performed on the data comparing specific binding levels in the lesioned and nonlesioned nucleus accumbens. A level of $P < .05$ was used to declare statistical significance for all analyses.

3. Results

3.1. Lesion results

Surgery was performed on 116 rats. Of these, 9 died before behavioral testing occurred due to complications from anesthesia or surgery. Of the remaining rats, 66 had dopamine depletion levels greater than 80%, and only these animals were used in the analyses.

3.2. Behavioral data

Fig. 1 presents the mean (±S.E.M.) number of contralateral and ipsilateral rotations for each group graphed at each time block. Treatment with amphetamine induced contralateral rotation, and this rotation was blocked by pretreatment with U-99194A. Later in the test session (10–20 and 20–30 min blocks), amphetamine induced ipsilateral rotation and pretreatment with U-99194A did not significantly alter this rotation. U-99194A pretreatment followed by saline appeared to induce low levels of ipsilateral rotation at each

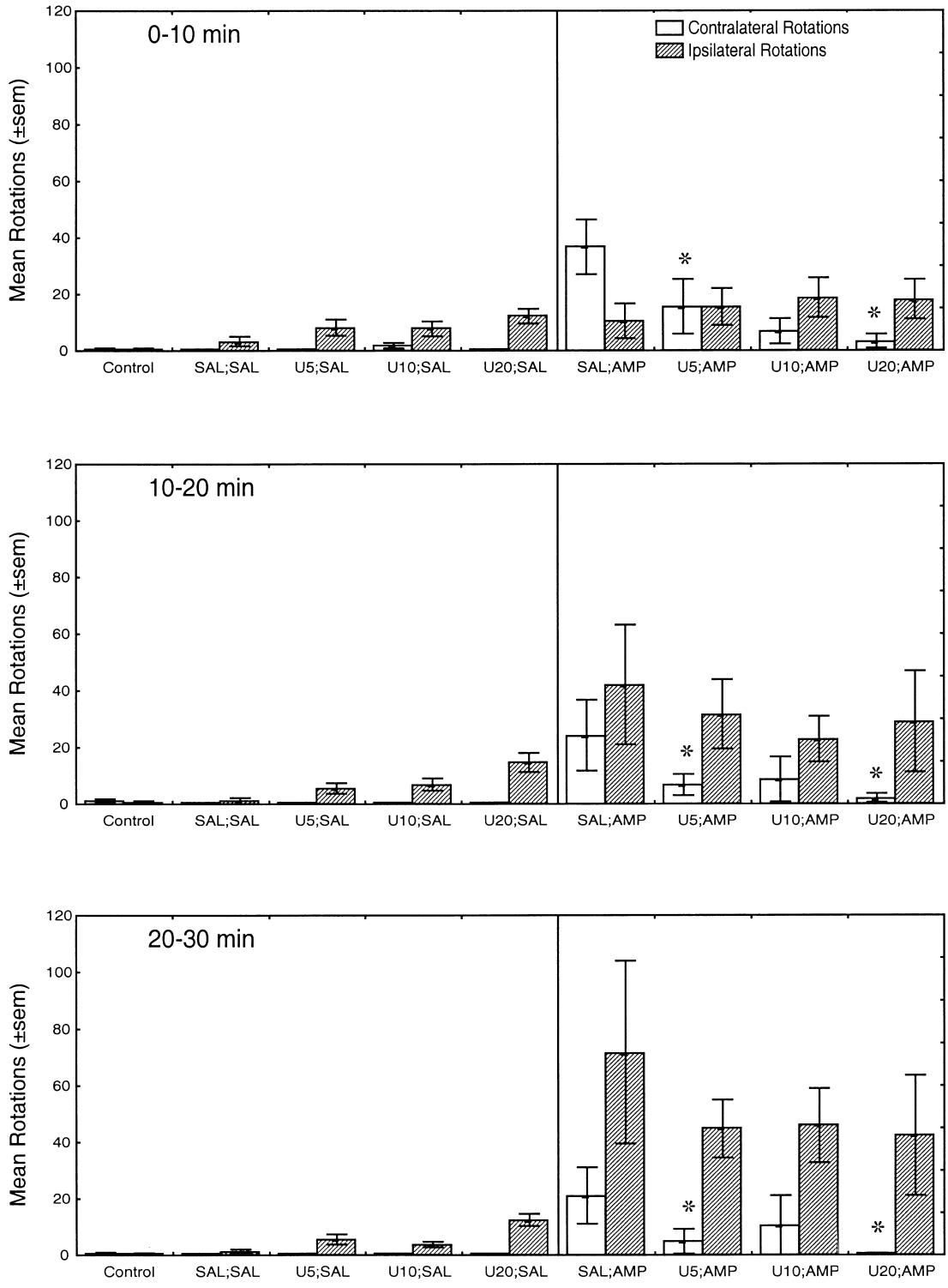

Fig. 1. Mean contralateral and ipsilateral rotations (±S.E.M.) 48 h following 6-OHDA lesion, graphed by group at each time block (0–10, 10–20 and 20–30 min). Contralateral rotation following amphetamine was blocked by 5 and 20 mg/kg U-99194A. Amphetamine-induced ipsilateral rotation was observed later in the test session and was not significantly altered by any dose of U-99194A. * Indicates significant decrease from SAL–AMP group. U5 = 5 mg/kg U-99194A; U10 = 10 mg/kg U-99194A; U20 = 20 mg/kg U-99194A.

dose tested; however, this rotation was not significantly higher than the saline/saline-treated rats.

The vehicle-injected control animals displayed almost no rotation in response to amphetamine (mean contralateral = 2.25 ± 1.11; mean ipsilateral = 1.75 ± 0.48). This is consistent with previous work examining rotation in vehicle-injected controls (Carey, 1992). The data from these control animals were not included in the statistical analyses but are represented in Fig. 1.

3.2.1. Contralateral rotation

The ANOVA performed on these data revealed significant main effects of pretreatment [$F(3,58) = 3.05$, $P < .05$] and group (amphetamine or saline) [$F(1,58) = 12.03$, $P < .01$], but no main effect of time ($P < .27$). A significant Pretreatment × Group interaction was found [$F(3,58) = 3.07$, $P < .05$]. There were no other significant interactions. Separate one-way ANOVAs were performed on the saline and amphetamine groups collapsed across time blocks to further analyze this interaction. The test for simple effects in the saline groups was not significant ($P < .23$). Pretreatment with 5 mg/kg and 20 mg/kg U-99194A significantly decreased contralateral rotation in response to amphetamine compared to saline pretreatment [$F(3,31) = 3.24$, $P < .05$; Newman–Keuls, $P < .05$ for both comparisons]. The analysis did not reveal a significant decrease in contralateral rotation for the 10 mg/kg dose of U-99194A ($P < .09$).

3.2.2. Ipsilateral rotation

The ANOVA performed on ipsilateral rotation revealed a significant main effect of group [$F(1,58) = 12.25$, $P < .01$] and time [$F(2,116) = 9.89$, $P < .01$], but not pretreatment ($P < .97$). The Group × Time interaction was significant [$F(2,116) = 12.29$, $P < .01$). To analyze this interaction, a separate ANOVA was performed at each time block. Simple effects at each time block revealed that ipsilateral rotation in the amphetamine groups increased over time compared to the saline groups [0–10 min: $F(1,64) = 5.80$, $P < .01$, 10–20 min: $F(1,64) = 9.94$, $P < .01$, 20–30 min: $F(1,64) = 18.22$, $P < .01$]. In addition, an ANOVA performed for each group revealed a significant effect of time block [$F(2,62) = 13.34$, $P < .01$] for amphetamine-treated rats but not saline-treated rats ($P > .05$), further supporting an increase in amphetamine-stimulated ipsilateral rotation over time.

3.3. Correlational analysis

A correlational analysis revealed a significant relationship between percent depletion of dopamine in the striatum and number of ipsilateral rotations in amphetamine-treated rats, ($r = .37$, $P < .05$). In addition, a significant inverse relationship was found between percent depletion of dopamine in the striatum and number of contralateral rotations in amphetamine-treated rats, ($r = -.39$, $P < .05$). Although a significant correlation was obtained, inspection of the data revealed an apparent "stair-step" function. That is, the incidence of ipsilateral rotation was relatively constant in rats having between 80% and 97% dopamine depletion, but increased dramatically at depletion levels of 98% and higher. In order to examine this difference in behavioral response, a median split analysis was performed based on the percent depletion of dopamine in the striatum. Animals whose depletion levels were at or above the median (98%) were grouped as ≥ 98% dopamine depletion ($n = 34$). Rats whose depletion levels fell below the median were grouped as 80–97% dopamine depletion ($n = 32$). Within each depletion level group, the number of animals in each treatment condition where as follows:

≥ 98% dopamine depletion

	Vehicle	Agonist tested
Antagonist pretreatment	Saline	Amphetamine (2.5 mg/kg)
Saline	$N = 5$	$N = 5$
U-99194A (5 mg/kg)	$N = 1$	$N = 7$
U-99194A (10 mg/kg)	$N = 3$	$N = 6$
U-99194A (20 mg/kg)	$N = 2$	$N = 5$

80–97% dopamine depletion

	Vehicle	Agonist tested
Antagonist pretreatment	Saline	Amphetamine (2.5 mg/kg)
Saline	$N = 6$	$N = 4$
U-99194A (5 mg/kg)	$N = 4$	$N = 3$
U-99194A (10 mg/kg)	$N = 6$	$N = 2$
U-99194A (20 mg/kg)	$N = 4$	$N = 3$

3.4. Behavioral data for ≥98% dopamine depletion rats

Fig. 2 represents the mean (± S.E.M.) number of contralateral and ipsilateral rotations for each group graphed at each time block for the ≥ 98% dopamine depletion rats. Only a small number of contralateral rotations were observed in this group, and these rotations showed no clear time-dependent change across the test session. In contrast, these animals exhibited more ipsilateral rotations, particularly during the last 20 min of testing. Pretreatment with U-99194A did not significantly alter ipsilateral rotation in these rats.

3.5. Contralateral rotation — ≥98% dopamine depletion rats

The ANOVA performed on contralateral rotations for the ≥ 98% dopamine depletion rats revealed no significant main effects or interactions. Thus, no further statistical analyses were performed on these data.

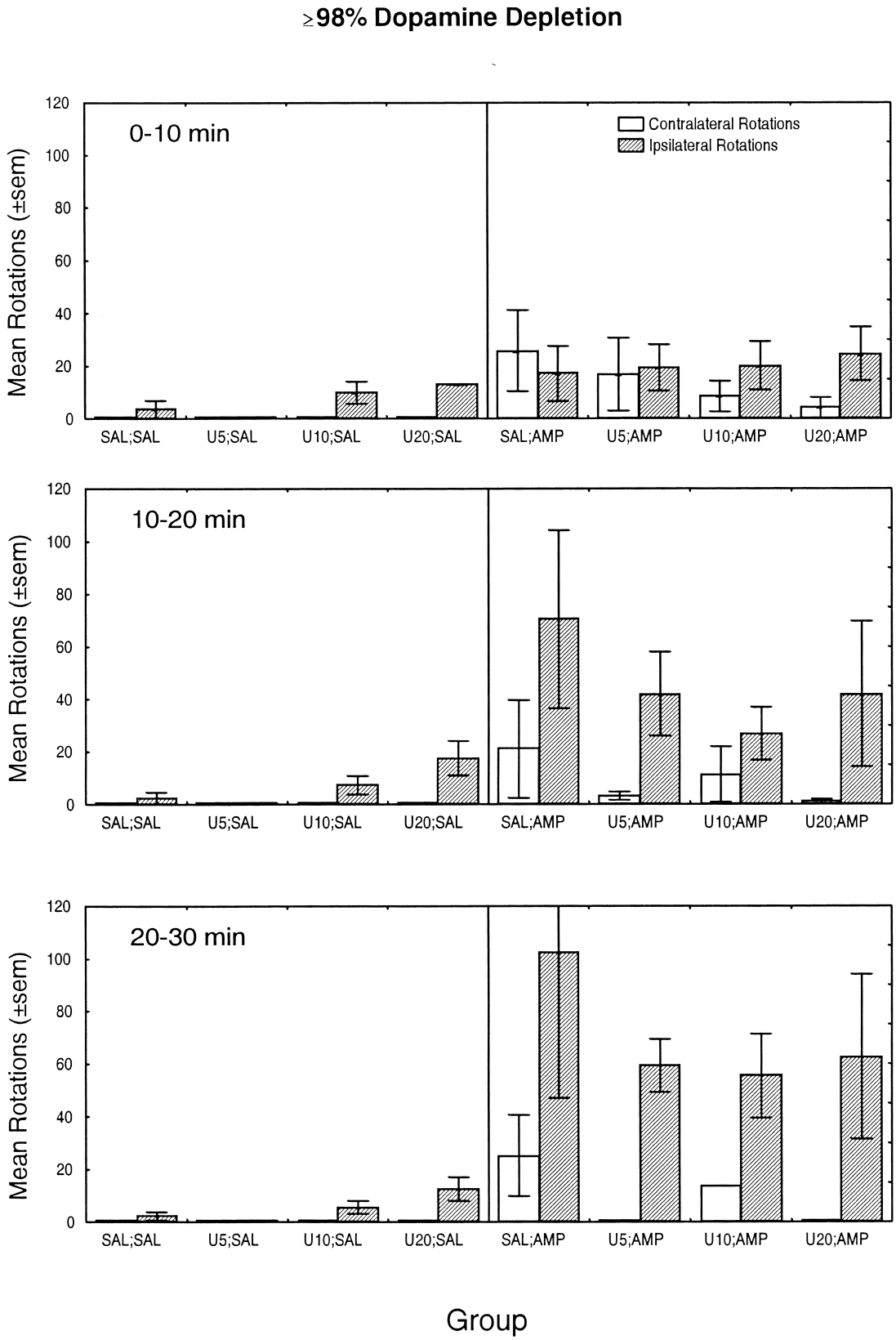

Fig. 2. Rats with ≥98% dopamine depletion. Mean contralateral and ipsilateral rotations (±S.E.M.) for the animals with ≥98% dopamine depletion 48 h following 6-OHDA lesion, graphed by group at each time block (0–10, 10–20 and 20–30). Ipsilateral rotation following amphetamine was increased at each time block compared to saline-treated rats. Pretreatment with U99194A did not significantly alter ipsilateral rotation at any dose tested. U5=5 mg/kg U-99194A; U10=10 mg/kg U-99194A; U20=20 mg/kg U-99194A.

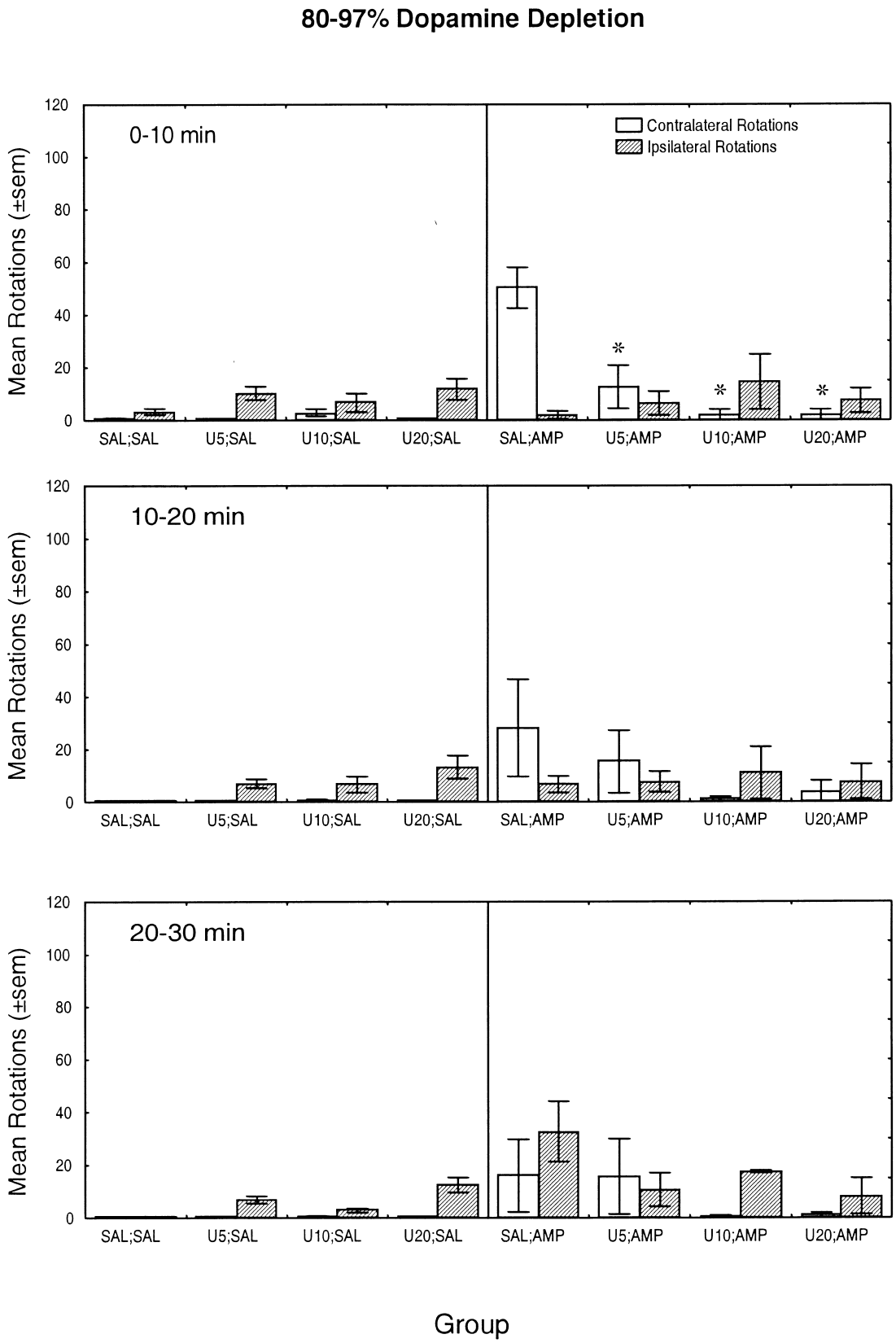

Fig. 3. Rats with 80–97% dopamine depletion. Mean contralateral and ipsilateral rotations (±S.E.M.) for the rats with 80–97% dopamine depletion 48 h following 6-OHDA lesion, graphed by group at each time block (0–10, 10–20 or 20–30 min). Pretreatment with U99194A decreased contralateral rotation in amphetamine treated rats, compared to saline pretreatment during the first 10 min of testing. * Indicates significant decrease from SAL–AMP group. U5 = 5 mg/kg U-99194A; U10 = 10 mg/kg U-99194A; U20 = 20 mg/kg U-99194A.

3.6. Ipsilateral rotation — ≥98% dopamine depletion rats

The ANOVA performed on ipsilateral rotations for the ≥98% dopamine depletion rats revealed a significant main effect of group [$F(1,26)=6.14$, $P<.05$] and time [$F(2,52)=4.18$, $P<.05$], but not pretreatment ($P<.9$). Only the Group × Time interaction was significant [$F(2,52)=4.68$, $P<.01$). To analyze this interaction, a separate ANOVA was performed at each time block. Simple effects at each time block revealed that ipsilateral rotation in the amphetamine group, regardless of pretreatment, increased over time compared to the saline group [0–10 min: $F(1,32)=4.33$, $P<.05$, 10–20 min: $F(1,32)=5.98$, $P<.05$, 20–30 min: $F(1,32)=9.53$, $P<.01$]. In addition, an ANOVA performed for each group revealed a significant effect of time block [$F(2,38)=12.57$, $P<.01$] for amphetamine-treated rats but not saline-treated rats ($P>.05$), further supporting an increase in amphetamine-stimulated ipsilateral rotation over time.

3.7. Behavioral data for 80–97% dopamine depletion rats

Fig. 3 represents the mean (±S.E.M.) number of contralateral and ipsilateral rotations for each group graphed at each time block for the 80–97% dopamine depletion rats. During the first 10 min of testing, these rats exhibited contralateral rotation that was blocked by pretreatment with U-99194A at all doses tested. By the last 10 min of testing, these animals exhibited some ipsilateral rotation. The amphetamine-induced ipsilateral rotation was not significantly altered by U-99194A pretreatment. However, the 5- and 20-mg/kg pretreatment doses of U-99194A enhanced ipsilateral rotation in the saline-treated rats during the last 10 min of testing.

3.8. Contralateral rotation — 80–97% dopamine depletion rats

The ANOVA performed on these data revealed significant main effects of pretreatment [$F(3,24)=3.59$, $P<.05$], group [$F(1,24)=9.08$, $P<.01$] and time [$F(2,48)=4.04$, $P<.05$]. The Pretreatment × Group × Time interaction was also significant [$F(6,48)=4.5$, $P<.01$]. To analyze this interaction, separate ANOVAS were performed at each time block. The Pretreatment × Group interaction was significant only at the 0–10 min time block [$F(3,24)=22.07$, $P<.01$]. The main effect of pretreatment [$F(3,24)=21.91$, $P<.01$] and group [$F(3,24)=34.96$, $P<.01$] were significant at this time block. The test for simple effects in the saline groups at the 0–10 min time block was not significant ($P<.13$). The test for simple effects in amphetamine groups was significant [$F(3,8)=12.63$, $P<.01$]. Post hoc analysis found that pretreatment with 5, 10, and 20 mg/kg U-99194A decreased contralateral rotation compared to pretreatment with saline (Newman–Keuls, $P<.01$ for comparisons).

3.9. Ipsilateral rotation — 80–97% dopamine depletion rats

The ANOVA performed on these data revealed a significant main effect of time [$F(2,48)=4.32$, $P<.05$], but not pretreatment ($P<.8$) or group ($P<.08$). The Pretreatment × Group × Time interaction was also significant [$F(6,48)=3.60$, $P<.01$]. To analyze this interaction, separate ANOVAS were performed at each time block. The Pretreatment × Group interaction was significant only at the 20- to 30-min time block [$F(3,24)=5.6$, $P<.01$]. The test for simple effects in the saline groups at the 20- to 30-min time block was significant [$F(3,16)=14.73$, $P<.01$], but the test for simple effects in amphetamine groups was not significant ($P<.25$). Post hoc analysis found that pretreatment with 5, and 20 mg/kg U-99194A induced ipsilateral rotation in the saline animals during the last 10 min of testing compared to pretreatment with saline (Newman–Keuls, $P<.05$ for both comparisons).

3.10. Binding assay

The dopamine levels on the lesioned striatum of the saline-treated animals were compared to the dopamine levels on the intact striatum and a percent depletion was calculated. In addition, the dopamine levels on the lesioned nucleus accumbens were compared to the dopamine levels on the intact nucleus accumbens. Only rats with at least 80% depletion in the striatum and a coinciding 50% depletion in the nucleus accumbens were used in the data analysis ($N=7$). Dopamine depletion in the striatum ranged from 82% to 96%. Dopamine depletion in the nucleus accumbens ranged from 52% to 76%.

The mean percent decrease in specific binding on the lesioned side, compared to the nonlesioned side, was 29.25±6.51%. The t test performed on these data revealed a significant decrease in D3 receptor binding on the lesioned side, [$t(6)=-3.82$, $P<.009$]. On the lesioned side, a mean of 0.44±0.06 fmol bound/mg wet weight tissue was obtained. On the nonlesioned side, a mean of 0.62±0.12 fmol bound/mg wet weight tissue was obtained. These results confirm that in our hands, a decrease in D3 receptor binding occurs 48 h after a 6-OHDA lesion of the medial forebrain bundle.

4. Discussion

The aim of the present experiment was to investigate the possibility that alterations in dopamine D3 receptors play a role in the early compensatory response of the dopamine system following a 6-OHDA lesion of the medial forebrain bundle. Animals were tested for their rotational response to amphetamine 48 h postlesion. This postlesion time interval was chosen because D2 receptors do not appear to be up-regulated (Narang and Wamsley, 1995; Neve et al., 1982),

obviating an imbalance in D2 receptor sensitivity as an explanation for any observed rotation. In contrast to D2 receptors, decreases in D3 receptors have been found at this time interval (Lévesque et al., 1995). Thus, the present experiment examined the effect of pretreatment with the D3 antagonist U-99194A on rotational behavior induced by amphetamine 48 h following injection of 6-OHDA.

As a whole, the rats treated with amphetamine exhibited both contralateral and ipsilateral rotations. During the first 10 min of testing, the rats in the saline/amphetamine group exhibited mostly contralateral rotation and this rotation was decreased by pretreatment with U-99194A (5 and 20 mg/kg). This suggests that amphetamine was acting to increase dopamine levels on both sides of the brain and that the lesioned side may have had an enhanced ability to release dopamine due to compensatory mechanisms (described above). This higher level of releasable dopamine on the lesioned side resulted in contralateral rotations being observed. In the rats treated with U-99194A, the inhibitory D3 receptors on both sides of the brain were blocked, thus restoring a balance in inhibition and allowing D2 receptors to control stimulation; essentially restoring the balance in stimulation between both sides of the brain.

By the last 10 min of testing, mostly ipsilateral rotation was observed in all of the amphetamine groups and this rotation was not altered by pretreatment with U-99194A at any dose tested. This suggests that amphetamine may deplete remaining terminals of dopamine over time, decreasing the effectiveness of compensatory mechanisms and resulting in the observation of ipsilateral rotations. U-99194A did not significantly induce rotation when given alone at any dose tested. These data support the hypothesis that D3 receptors have a role in amphetamine-induced contralateral rotation following a 6-OHDA lesion of the medial forebrain bundle.

While the procedure for the 6-OHDA lesions was the same for each animal, the outcome of the lesion was not. Some lesions resulted in extensive depletion of dopamine in the basal ganglia ($\geq$98%), while others resulted in relatively less extensive depletions (80–97%). This level of depletion was correlated with the direction of amphetamine-stimulated rotational behavior. Rats with $\geq$98% depletion levels were more likely to rotate in the ipsilateral direction and less likely to rotate in the contralateral direction. Thus, there was a qualitative difference in the behavioral response of the animals that depended on the extent of the lesion. This finding was the basis for examining the rats as two groups, rather than one, based on their depletion levels. Examining the data in this manner may allow for a more accurate and interesting test of the present hypothesis.

The finding that animals with $\geq$98% dopamine depletion are more likely to rotate in the ipsilateral direction is consistent with the original rotation model as developed by Ungerstedt (1971). That is, because the lesioned side contains less than 2% of the dopamine found in the nonlesioned side, dopamine is not released following amphetamine in sufficient concentrations to stimulate existing dopamine receptors. Therefore, only the nonlesioned side is stimulated, resulting in rotation ipsilateral to the lesion.

In contrast, in rats with 80–97% dopamine depletion, compensatory pre- and postsynaptic mechanisms may increase the availability and effectiveness of releasable dopamine on the lesioned side of the brain. In response to amphetamine, the lesioned side is stimulated at higher levels due to these spared compensatory mechanisms, and this results in rotation contralateral to the lesion.

In general then, ipsilateral rotation is mediated by more stimulation on the nonlesioned side while contralateral rotation is mediated by more stimulation on the lesioned side. The expressed rotational behavior, regardless of the direction, is thought to be mediated by the stimulatory postsynaptic D2 receptors on the side of the brain that is more stimulated (Schwarting and Huston, 1996; Ungerstedt, 1971). However, inhibitory D3 receptors may also have a role.

In the rats with $\geq$98% dopamine depletion, amphetamine induced mostly ipsilateral rotation, especially during the last 20 min of testing. This is consistent with the idea that amphetamine is acting on the nonlesioned side to release dopamine, resulting in more stimulation on the nonlesioned side, and consequently, ipsilateral rotation (Schwarting and Huston, 1996; Ungerstedt, 1971). Pretreatment with U-99194A did not block amphetamine-induced ipsilateral rotation in these animals.

In animals with 80–97% dopamine depletion, contralateral rotation was observed, especially during the first 10 min of testing. As testing progressed, the rats began to show some ipsilateral rotation. This is consistent with the idea that amphetamine may deplete releasable pools of dopamine on the lesioned side during the first 10–20 min following injection, resulting in a switch from contralateral to ipsilateral rotation during the 30 min test (Robinson and Whishaw, 1988; Schwarting and Huston, 1996). Pretreatment with U-99194A blocked amphetamine-induced contralateral rotation almost completely within the range of doses tested (5–20 mg/kg), suggesting that D3 receptors are involved in amphetamine-induced contralateral rotation observed 48 h following injection of 6-OHDA. This effect did not appear to be dose-dependent within the range of doses tested.

Based on the present hypothesis, the results of this study suggest that U-99194A is acting to block the inhibitory effect of D3 receptor stimulation on both sides of the brain. Following treatment with amphetamine, both sides of the brain are being activated via the stimulatory D2 receptors. Thus, there is no imbalance in stimulation, and consequently, little rotation in either direction.

It could also be argued that U-99194A is acting mostly at D2 receptors to decrease contralateral rotation. However, this is unlikely because if U-99194A is acting to decrease contralateral rotation via D2 receptors, then it should decrease both contralateral and ipsilateral rotation equally

because it would be doing so via the same mechanism. That is, at 48 h postlesion, the density of D2 receptors is similar on each side of the brain (Neve et al., 1982; Ungerstedt, 1971). Depending on which side of the brain is more stimulated by amphetamine (based on depletion levels), the D2 receptors on that side would mediate the rotation. If D2 receptors are blocked, then rotation in either direction would be minimized. In contrast, based on the present hypothesis, blockade of D3 receptors would result in a decrease in contralateral rotation, but not ipsilateral rotation. The decrease in contralateral rotation would be due to the D2 receptors on the nonlesioned side being released from the inhibitory effect of D3 stimulation, resulting in equal stimulation on each side via the D2 receptors. With ipsilateral rotation, this same blockade on the nonlesioned side would result in D2 receptors still being stimulated and no decrease in ipsilateral rotation would be expected. Indeed, an increase in amphetamine-induced ipsilateral rotation would be predicted following D3 receptor blockade because the D2 receptors on the intact side would be released from the inhibitory effect of D3 receptors. However, this was not the case in the present experiment. The failure of U-99194A to increase ipsilateral rotation in the present results may have been due to the high rate of rotation induced by 2.5 mg/kg amphetamine such that further enhancement of rotation may have been difficult to detect. Further studies using varying doses of amphetamine are necessary to test this hypothesis.

Taken together, the results from the present experiment support the hypothesis that the decrease in D3 receptors seen following denervation is involved in the compensatory response of the dopamine system. It should be considered, however, that the sample size in each treatment group was small after the median split analysis based on depletion levels was performed. While statistically significant, the number of rats in each cell was quite small. However, the goal of creating two groups based on depletion levels was to further examine the statistically significant differences found when the animals were examined as a whole. By examining the animals based on depletion levels, we were able to see the differences that account for the significant results when the lesioned animals were analyzed as a whole group. Following an injection of 6-OHDA, it is not possible to insure a particular level of dopamine depletion in each rat. The level of depletion can vary due to individual differences among the animals. Varying the dose of 6-OHDA, however, may more reliably create animals with different depletion levels. These types of experiments are necessary to further test the present hypothesis.

It should also be considered that the pharmacologic tools used in this experiment are still being evaluated for their selectivity. While the drug U-99194A has been found to possess a 20-fold greater affinity for the D3 receptor over the D2 receptor (Kling-Petersen et al., 1995; Waters et al., 1993), further evaluation of the ability of this compound to specifically interact in vivo with D3 receptors is necessary. It is often the case that a particular compound may possess multiple in vivo properties that are coupled to the dose range of the compound (Kenakin, 1993). Further, in some cases, it is the multiple properties of a particular drug that account for its behavioral effect.

Elucidation of the role of D3 receptors in the compensatory response to denervation may have important clinical relevance in the treatment of neuropsychiatric disorders involving the basal ganglia. In contrast to the D2 receptor, few D3 receptors are located in brain areas (dorsal striatum) that are associated with extrapyramidal side effects of pharmacotherapies (e.g., Bancroft et al., 1998; Levant, 1998). Instead, the D3 receptor is more densely distributed in areas associated with the motivational properties of stimuli, i.e., the nucleus accumbens (Pontieri et al., 1995; Wise, 1996). This suggests that the D3 receptor may be a novel target for the treatment of disorders such as Parkinsonism and drug abuse (Sokoloff et al., 1990).

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