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Rats with unilateral median forebrain bundle, but not striatal or nigral, lesions by the neurotoxins MPP^+ or rotenone display differential sensitivity to amphetamine and apomorphine

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Abstract

Rotenone and 1-methyl-4-phenyl pyridinium (MPP^+) are two mitochondrial neurotoxins known to produce Parkinson's disease (PD) in experimental animals. In the present study, we compared drug-induced rotational asymmetry in rats lesioned using these neurotoxins at three distinct basal ganglia sites, the striatum, substantia nigra pars compacta (SNpc) and median forebrain bundle (MFB). The levels of dopamine (DA) in the ipsilateral striata of these hemiparkinsonian animals were assayed employing an HPLC-electrochemical procedure 2 days after the final rotational study. Rats infused with rotenone or MPP^+ into the SNpc, but not into the striatum or MFB, exhibited contralateral rotations immediately after recovery from anesthesia. Irrespective of the lesion site or the toxin used, all the animals exhibited ipsilateral rotations when challenged with *d*-amphetamine. Apomorphine administration caused contralateral circling behavior in MFB-lesioned animals, but ipsilateral rotations in rats that received rotenone or MPP^+ in the striatum or SNpc. Stereotaxic administration of rotenone into the MFB, SNpc or striatum caused a significant loss of DA in the ipsilateral striatum to varying degrees (96%, 62% and 30%, respectively, as compared to the contralateral side). However, unilateral MPP^+ administration into the MFB, SNpc or striatum caused respectively about 98%, 74% and 59% loss of striatal DA. Behavioural observations and the neurochemical results indicate that, among the three anatomically distinct loci-lesioned, MFB-lesioned animals mimicked behavioral aberrations similar to nigral lesions caused by 6-hydroxydopamine, a classical parkinsonian neurotoxin. Moreover, the results point out that while both *d*-amphetamine and apomorphine-induced rotations could be considered as valuable behavioral indices to test novel drugs against PD, yet apomorphine-induced contralateral bias proves to be a more reliable indicator of specific destruction in the nigrostriatal pathway and development of post-synaptic DA receptor supersensitivity.

Keywords: Circling behavior; Complex-I inhibitor; animal model; Drug screening; Dopamine receptor supersensitivity; Parkinson's disease; MPP^+; 6-OHDA; Rotenone; Contralateral and ipsilateral rotations

1. Introduction

In Parkinson's disease (PD) research, experimental animal models have contributed immensely to the understanding of the pathophysiology of the disease and for designing therapeutic strategies. Even though both unilaterally and bilaterally lesioned rodents and primates are extensively used in PD research, the latter group resembles human PD in terms of biochemistry. However, unilaterally lesioned models provide greater advantages as they produce a range of quantifiable behavioral abnormalities, which can be harnessed for screening drugs and testing efficacy of transplants. Lesion on one side of the brain manifests in several spontaneous

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behavioral alterations (Schwarting et al., 1991; Sindhu et al., 2005) as well as drug-induced rotations, which serve as a litmus test for lesion confirmation. In this regard, the striatum, substantia nigra pars compacta (SNpc) and median forebrain bundle (MFB) are the three commonly used anatomical loci for neurotoxin administration. However, the ideal target site for administration of a particular neurotoxin in the brain for producing lesions that mimic parkinsonism in animals remains ambiguous.

6-Hydroxydopamine (6-OHDA) is the most common neurotoxin that has been used for producing site-specific lesions for the development of experimental PD. This dopaminergic neurotoxin induces many of the parkinsonian symptoms in rodents. A major breakthrough in PD research has been the discovery of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) that causes parkinsonism in humans and non-human primates (Langston et al., 1983; Kopin and Markey, 1988). Upon systemic administration, MPTP crosses through the blood–brain barrier into the brain, where it is metabolized to 1-methyl-4-phenylpyridinium ion (MPP^+) by the enzyme monoamine oxidase-B (Cohen et al., 1984; Heikkila et al., 1984; Mitra et al., 1994). MPP^+ is taken up through dopamine (DA)-transporters into DA-ergic neurons (Mayer et al., 1986; Jourdain et al., 2005), where it triggers cell death through complex-I inhibition (Nicklas et al., 1985; Mizuno et al., 1987).

Mitochondrial complex-I inhibitor, rotenone, is a potent parkinsonian neurotoxin that can reproduce several neurochemical, behavioral and neuropathological features of PD, including Lewy body formation following its chronic, systemic administration (Betarbet et al., 2000; Alam and Schmidt, 2002, 2004; Alam et al., 2004). Acute intracranial single-time administration of rotenone has been shown to cause a slow, but progressive degeneration of the striatal DA-ergic pathway, resulting in biochemical lesions resembling idiopathic PD (Saravanan et al. 2001, 2005). Unilateral intranigral or intra-median forebrain bundle infusion of rotenone has also been shown to develop neurochemical imbalance in the basal ganglia (Heikkila et al., 1985; Sindhu et al., 2005) resulting in behavioral asymmetry in rats (Sindhu et al., 2005).

In 6-OHDA-induced hemiparkinsonian model *d*-amphetamine-induced ipsiversive turnings and apomorphine-mediated contralateral bias are well documented (Ungerstedt and Arbuthnott, 1970; Ungerstedt, 1971; Hudson et al., 1993; Schwarting and Huston, 1996). The latter effect has been attributed to supersensitivity developed to the post-synaptic DA receptors in the striatum following long-term extensive DA depletion. While a couple of studies reported contraversive circling behaviour in rats intranigrally infused with MPP^+ (Lange, 1989, 1990), others reported ipsiversive circling after the administration of apomorphine (Sun et al., 1988; Kondo et al., 2004). However, animals that received unilateral infusion of MPP^+ into MFB depicted contralateral circling in response to apomorphine (Sirinathsinghji et al., 1988, 1990). Except for our own recent study (Sindhu et al., 2005), there is no other report on rotenone's rotational asymmetry. An isolated study reported non-responsiveness of animals to apomorphine after a single small dose of rotenone in SNpc (Kondo et al., 2004).

In our investigations, we observed that intranigrally MPP^+- or rotenone-infused animals displayed ipsilateral rotations to apomorphine administration, which is contrary to the pattern shown in 6-OHDA-lesioned animals. This unexpected rotational behavior to apomorphine in nigrally lesioned rats prompted us to examine the effects of these toxins in different loci in the basal ganglia circuitry, which has never been attempted. We expected both rotenone and MPP^+ to behave similarly since they share the same biochemical mechanism of action in the brain, and thus we compared the behavioral deficits to see whether there exists any variation when injury is made in three different locations in the DA-ergic nirgostriatal pathway. In the present study, we report for the first time apomorphine-induced contralateral rotations in unilaterally MPP^+- or rotenone-lesioned rats following intracranial infusion into MFB, but not into striatum or SNpc.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats (250–300 g) from the institute colony were used in the present study. The animals were maintained under standard conditions of 12 h light/dark cycles, 22±1 °C temperature and 60±5% humidity. They were provided food and water ad libitum. The experimental protocols met with the national guidelines on the "Proper Care and Use of Animals in Laboratory Research" (Indian National Science Academy, New Delhi, 2000) and were approved by the animal ethics committee of the institute.

2.2. Drugs and chemicals

Rotenone, MPP^+, 6-OHDA, DA, apomorphine, *d*-amphetamine sulphate and EDTA were procured from Sigma Chemicals (St. Louis, MO). Chloral hydrate was obtained from Fluka, Germany. Acetonitrile, heptane sulfonic acid, *O*-phosphoric acid, triethylamine and perchloric acid used for HPLC analysis were procured from SISCO Research Laboratories, Mumbai, India. *d*-Amphetamine and apomorphine were dissolved respectively in double distilled water and in 1% sodium metabisulphite.

2.3. Experimental design

Rats were divided into 10 groups. The first three groups received rotenone dissolved in DMSO and the 4th–6th groups received MPP^+ intranigrally, intrastriatally and intra-MFB. The 7th–9th groups received the vehicle (DMSO) in these three regions. The last group of animals received 6-OHDA intranigrally. This group served as a positive control.

Rats were tested for amphetamine-response on 14th and apomorphine-response on 16th day. In the case of rotenone–MFB group, this time schedule was extended till the 28th and 30th days respectively since these animals when checked on 14th and 16th days showed very low number of rotations to *d*-amphetamine and apomorphine respectively (see Sindhu et al.,

2005 for details). Two days after the last rotational study, the animals were sacrificed and the striatal DA was measured employing a sensitive HPLC-electrochemical procedure.

2.4. Surgery

Rats were anesthetized with chloral hydrate (450 mg/kg, i.p.). The animal was placed in the flat skull position on a cotton bed on a stereotaxic frame (Stoelting, USA) with incisor bar fixed at 3.5 mm below the interaural line. Rotenone dissolved in DMSO (6 μg in SNpc and 12 μg in MFB, both in 1 μl) or MPP^+ dissolved in saline (19 μg in 1 μl) was infused into the right SNpc or MFB at a flow rate of 0.2 μl/min employing a microinfusion pump consisting of a Worker Bee and Syringe Pump (BAS, West Lafayette, USA). In the case of striatal infusion, the flow rate was 0.5 μl/min for 8 min (in a total volume of 4 μl, 12 μg for rotenone and 29.7 μg for MPP^+). After stopping the infusion of the toxin, the probe was kept in the same position for a further 5 min for complete diffusion of the drug and then slowly retracted. The stereotaxic coordinates for SNpc were: lateral (L)=+0.20 cm, antero-posterior (AP, from the bregma point)=−0.53 cm and dorsi-ventral (DV)=+0.75 cm; for MFB: L=+0.15 cm, AP=−0.28 cm (from the bregma point) and DV=+0.80 cm; and for the striatum: L=+0.26 cm, AP=−0.02 cm (from the bregma point) and DV=+0.45 cm (Paxinos and Watson, 1998). Proper postoperative care was provided till the animals recovered completely.

2.5. Spontaneous circling behavior

Animals were observed for any spontaneous behavioral abnormalities during the first three days. One of the striking features following recovery from anesthesia was spontaneous circling (360° in a short axis) behavior in nigrally lesioned group. The animals were kept in a transparent cage (45 cm diameter and 40 cm height) 24 h following surgery and the spontaneous rotations in the cage were counted by trained/experienced individuals for 30 min.

2.6. Drug-induced rotations

Rats were placed in Perspex, transparent spherical cages (45 cm wide and 40 cm deep) and the rotations were counted for all the groups after *d*-amphetamine or apomorphine injections. Rotations (360°, in short axis) ipsilateral and contralateral to the side of infusion were counted by individuals trained in behavioral observation. Rats were tested following response to amphetamine (5 mg/kg, i.p.) on the 14th day. Two days later, the animals were administered apomorphine (1 mg/kg, s.c.). (We used 1.0 mg/kg of apomorphine in all the cases, since rats lesioned following intranigral infusion of rotenone failed to elicit rotational bias when treated with a lower apomorphine dose, 0.5 mg/kg). The number of ipsi- and contralateral rotations elicited was recorded from the initiation of rotational bias till the rotations vanished completely. Rotenone–MFB group showed very low number of rotations on 14th and 16th days respectively to *d*-amphetamine and apomorphine challenge. However, the ipsilateral bias to *d*-amphetamine and contralateral bias to apomorphine were evident in these rats, and they showed significant rotational bias as a response to the DA-ergic drugs on the 28th and 30th days post-lesion.

2.7. Determination of striatal DA levels

Animals were sacrificed two days after apomorphine treatment (18th day post-lesion in all the groups except in rotenone–MFB, where the rats were sacrificed on the 32nd day; see Sindhu et al., 2005 for results with 12 μg/animal intranigral or intra-MFB results). The left and right striata were micro-punched (Palkovits and Brownstein, 1983) separately and processed for the analyses of DA employing an HPLC-electrochemical procedure (Muralikrishnan and Mohanakumar, 1998). The tissue was sonicated in ice-cold 0.1 M $HClO_4$ containing 0.01% EDTA. The supernatant collected after centrifugation at 10,000×*g* for 5 min was injected (10 μl) into the HPLC system (Merck Hitachi, Germany) equipped with LaChrome L-3500A amperometric detector (Merck Hitachi, Germany) and C18, ion pair, analytical column (4.6×50 μm; Ultrasphere IP; Beckman, USA), with a particle size of 5 mm and pore of 80 Å. The flow rate was 0.7 ml/min and the electrochemical detection was performed at 0.74 V. The composition of the mobile phase was 8.65 mM heptane sulfonic acid, 0.27 mM EDTA, 13% acetonitrile, 0.43% triethylamine and 0.22% *O*-phosphoric acid.

2.8. Statistical analysis

Statistical analysis was performed using the Sigmastat-version 3 software. The rotational and neurochemical data were statistically evaluated for significance employing one-way ANOVA followed by Newman–Keuls post-hoc analysis. Results are given as mean±S.E.M. Values of $p \leq 0.05$ were considered significant.

3. Results

3.1. Spontaneous rotational behavior

After recovery from anesthesia, all the rats infused with rotenone or MPP^+ into SNpc exhibited spontaneous rotations contralateral to the side of infusion. The spontaneous rotations for rotenone and MPP^+ treated rats were 66±3.3 for 30 min duration at 24 h. The intensity of this behavior persisted over a day, slowly declined thereafter and vanished by the 3rd day. However, animals that received rotenone or MPP^+ in the striatum or MFB did not exhibit any spontaneous rotation.

3.2. Amphetamine-induced rotations

In MPP^+ treated group, *d*-amphetamine treatment on the 14th day post-lesion induced ipsilateral circling in nigral, striatal and MFB-lesioned groups (Fig. 1A–C). The rotenone-infused animals lesioned in SNpc or the striatum only responded to *d*-amphetamine displaying ipsilateral rotations on the 14th day

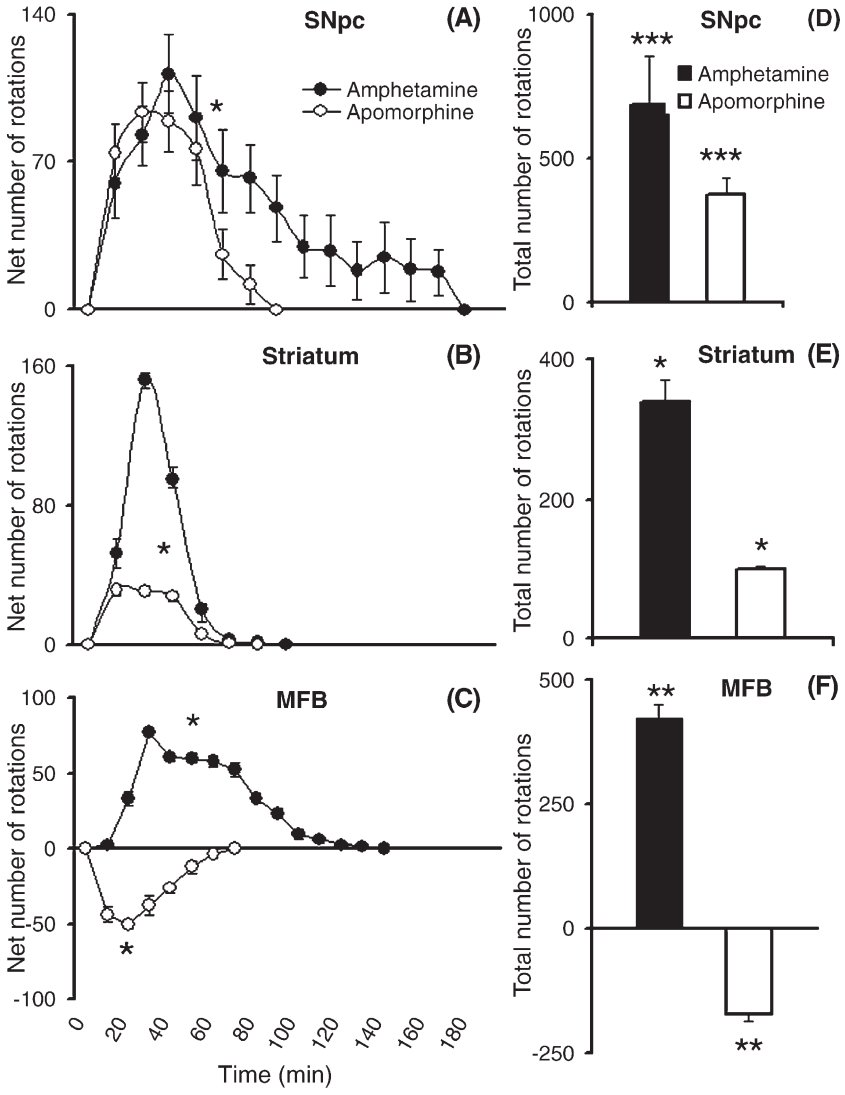

Fig. 1. Amphetamine- and apomorphine-induced rotational response in MPP^+-infused rats. Animals were unilaterally infused with MPP^+ (19 μg in 1 μl) or saline in substantia nigra pars compacta (SNpc) or in median forebrain bundle (MFB). Striatally infused rats received 29.7 μg of MPP^+ in 4 μl. These animals were injected with amphetamine (5 mg/kg, i.p.) on the 14th day and with apomorphine (1 mg/kg, s.c.) on the 16th day. Amphetamine- and apomorphine-induced rotations were recorded for every 10 min till the rotations became non apparent and scored for total number of rotations right from the beginning till cessation of rotations in each group. Positive values in the graph denote ipsilateral circling and negative values represent contralateral circling. Data expressed as rotations/10 min are depicted as mean ± S.E.M. *$p \leq 0.05$ as compared to control group ($n=6$), at each time point. Rotational scores have been expressed as total number of rotations for the entire period of response. *$p \leq 0.03$; **$p \leq 0.007$; ***$p \leq 0.001$ significantly different as compared to the control animals. Control animals infused unilaterally with saline showed no rotational bias.

(Fig. 2A,B). Unlike the SNpc or striatal lesions, intra-MFB rotenone-lesioned rats exhibited significant circling when challenged with *d*-amphetamine only on the 28th day post-lesion (Fig. 2C). Mean number of rotations for every 10 min is depicted in Figs. 1 and 2. The total number of ipsilateral rotations for the whole duration following *d*-amphetamine administration in MPP^+ and rotenone groups is depicted in Figs. 1D–F and 2D–F, respectively. Even though both rotenone and MPP^+ groups showed a similar bias in rotation, total number of rotations and duration of rotations varied. In 6-OHDA group, administration of *d*-amphetamine elicited ipsilateral rotations on the 14th day (Fig. 4A,C). Control groups with vehicle infusion did not show any rotational bias (values being 'zero' are not plotted in the graphs).

3.3. Apomorphine-induced rotations

Apomorphine injection on the 16th day post-lesion elicited significant circling behavior towards ipsilateral side in MPP^+ (Fig. 1A,B)- and rotenone (Fig. 2A,B)-infused SNpc and striatal groups. While MPP^+-MFB and 6-OHDA-SNpc group displayed contralateral rotations on the 16th day to apomorphine (Figs. 1C and 4A), rotenone–MFB group exhibited contralateral rotations to the DA agonist only on the 30th day (Fig. 2C). Mean number of rotations for every 10 min is depicted in Figs. 1 and 2. Total number of rotations for the entire period of rotational response for MPP^+ and rotenone is depicted in Figs. 1D–F and 2D–F. Total number of rotations for 6-OHDA treated group is shown in Fig. 4C. Control groups with vehicle infusion

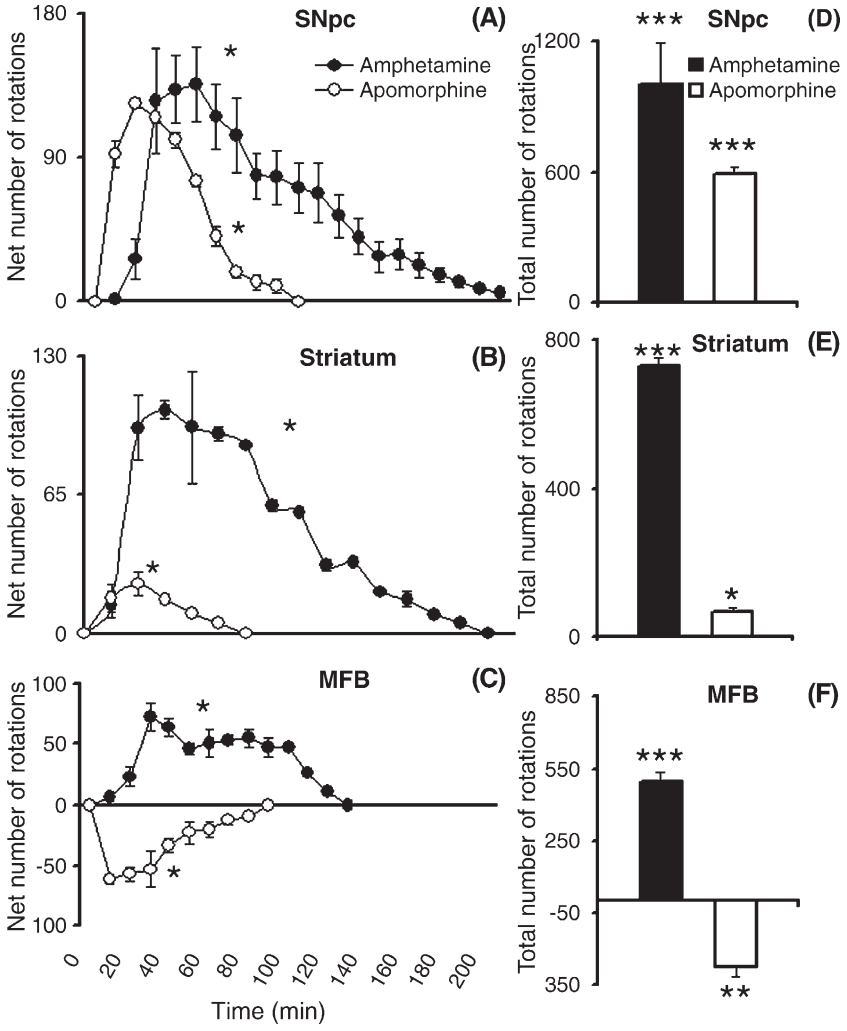

Fig. 2. Amphetamine- and apomorphine-induced rotational response in rotenone-infused rats. Animals were unilaterally infused with rotenone (6 μg for SNpc, and 12 μg for MFB in 1 μl DMSO, 12 μg in 4 μl DMSO for striatum) or vehicle in substantia nigra pars compacta (SNpc) or median forebrain bundle (MFB) or striatum. Intranigrally and intrastriatally rotenone-infused animals were injected with amphetamine (5 mg/kg, i.p.) on the 14th day and with apomorphine (1 mg/kg, s.c.) on the 16th day. Intra-MFB rotenone-infused animals were administered amphetamine (5 mg/kg, i.p.) on the 28th day and with apomorphine (1 mg/kg, s.c.) on the 30th day, since they exhibited no or very little rotational behavior on the 16th day. Amphetamine- and apomorphine-induced rotations were recorded till the rotations stopped completely and scored for total number of rotations right from the beginning till cessation of rotations in each group. Positive and negative values in the graph denote ipsilateral and contralateral rotations, respectively. Data expressed as number of rotations/10 min are depicted as mean±S.E.M. *$p \leq 0.05$ as compared to control group ($n=6$), at each time point. Total number of rotations for the entire period of circling response is represented as mean±S.E.M. ($n=6$). *$p \leq 0.05$; **$p \leq 0.003$; ***$p \leq 0.001$ significantly different as compared to control animals. Control animals infused unilaterally with DMSO showed no rotational bias.

did not show any rotational bias (values being 'zero' are not plotted in the graphs).

3.4. Effect of MPP^+, rotenone and 6-OHDA on striatal DA

Intranigral infusion of MPP^+ (64 nmol in 1 μl) resulted in 75% DA reduction in the ipsilateral striatum compared with the contralateral striatum on the 18th day. Striatal and MFB infusions of MPP^+ resulted in about 59% and 98% striatal DA depletion, respectively (Fig. 3A). Intranigral and intrastriatal infusions of rotenone caused a decrease of 62% and 30% in the striatal DA levels respectively on 18th day (Fig. 3B). Intra-MFB infusion caused 96% loss of DA in the ipsilateral striatum 32 days after rotenone administration as compared to the striatum contralateral to the side of infusion (Fig. 3B). The 6-OHDA-infused group showed 80% striatal DA depletion on the 18th day analysis (Fig. 4B).

4. Discussion

The most striking feature of the present study is difference in the rotational behavior in response to DA-ergic drugs following unilateral infusion of MPP^+ or rotenone in three different sites within the nigrostriatal pathway. Lack of contralateral rotational asymmetry in response to apomorphine in both intranigrally and intrastriatally MPP^+- or rotenone-lesioned animals and elicitation of contralateral bias to the administration of apomorphine in animals, which received MPP^+ or rotenone into MFB, are the important findings in the present study. Moreover, there is a clear dominance of neurotoxicity visible in intra-MFB animals

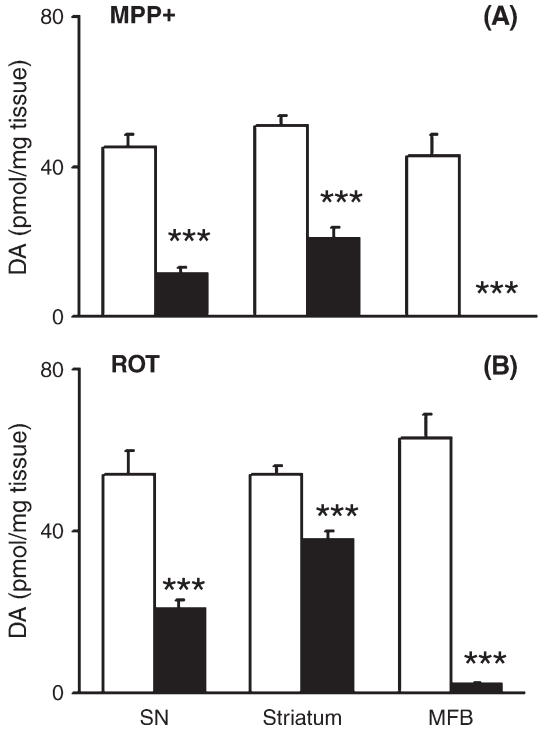

Fig. 3. Effect of intracranial infusion of MPP^+/rotenone at different loci on the striatal dopamine level. (A) Animals administered with MPP^+ either intranigrally (19 μg in 1 μl) or intrastriatally (29.7 μg in 4 μl) or intra-median forebrain bundle (MFB, 19 μg in 1 μl) were sacrificed on 18th day post-infusion, and analyzed for dopamine (DA) levels individually in the striata ipsi- or contralateral to the infused side by HPLC-electrochemistry. (B) Animals which received rotenone either intranigrally (6 μg in 1 μl) or intrastriatally (12 μg in 4 μl) were sacrificed on the 18th day post-infusion and those which received the toxin intra-median forebrain bundle (MFB, 12 μg in 1 μl), were sacrificed on the 32nd day post-infusion and analyzed for dopamine (DA) levels individually in the striata, ipsi- or contralateral to the infused side by HPLC-electrochemistry. Striatum was micropunched separately from left and right sides and were assayed for DA levels employing HPLC-electrochemistry. Two days after testing the apomorphine response (i.e., on the 32nd day) MFB-infused animals were sacrificed and analyzed for striatal DA content. ***$p \leq 0.001$ significantly different as compared to control animals. Data represent mean ± S.E.M. ($n = 6$ in each group).

over the intra-SN and intra-striatal models in regard to a total striatal DA loss.

Basal ganglia structures like caudate nucleus, globus pallidus and SN may differentially modulate behavior because of complex organization and interlinks. Animals with damage to any of the basal ganglia components or defect in the outgoing axonal bundles will show detectable motor impairment. Administration of DA releaser, *d*-amphetamine in hemiparkinsonian models activates the striatum contralateral to the side of infusion more than the damaged side and forces the animals to move towards the lesioned ipsilateral side (Schwarting and Huston, 1996). DA agonist apomorphine induces contralateral rotations in these animals because of the denervation-induced supersensitivity (Ungerstedt, 1971).

All the MPP^+-infused animals, in our present study irrespective of the site of injection of the neurotoxin, exhibited ipsilateral rotations after amphetamine challenge on the 14th day post-infusion. The variations in terms of total count, peak values and duration in amphetamine-induced rotations may indicate the dependency of the severity of striatal lesions following MPP^+ injections along the nigrostriatal pathway, since striatum is known to be involved in limb motor control (Pisa, 1988). However, very limited behavioral studies are available in rotenone-model (Alam and Schmidt, 2004; Alam et al., 2004; Fleming et al., 2004). Only one recent report exists in literature on the rotational asymmetry following rotenone-induced unilateral damage (Sindhu et al., 2005). The present study is the first report comparing rotenone's behavioral deficits with that of MPP^+ induced abnormalities.

The ipsilateral rotations to the administration of *d*-amphetamine indicate activation of the contralateral striatum and in turn suggest loss of DA in the striatum ipsilateral to the side of infusion. In this study, about 59%, 75% and 98% striatal DA depletion was obtained on the 18th day respectively following intrastriatal, intranigral and intra-MFB infusion of MPP^+. Rotenone caused 30%, 62% and 96% DA depletion respectively following striatal and nigral lesions on the 18th day, and MFB lesions on the 32nd day. Irrespective of the days of analysis, comparing the DA loss in the striatum on the day of experiment, it may be concluded that the behavioral manifestations result from imbalance in the striatal DA content and a mere 30% neurotransmitter depletion could elicit circling behavior in rats following amphetamine treatment.

Interestingly, in the present study, intranigrally as well as intrastriatally MPP^+- or rotenone-infused animals challenged with apomorphine exhibited ipsilateral rotations on the 16th day. This was contrary to that observed following striatal denervation caused by intranigral infusion of 6-OHDA (Ungerstedt and Arbuthnott, 1970; Schwarting and Huston, 1996). In the present study, 6-OHDA-induced nigral lesion elicited contralateral rotations to apomorphine insult. Thus, it is clear that the mechanisms of these parkinsonian toxins vary. Intrastriatal infusion of MPP^+ or rotenone caused ipsilateral rotations, which are similar to the circling behavior observed following apomorphine administration in animals intrastriatally infused with quinolinic acid (Ghorayeb et al., 2002). However, they failed to detect any rotation following apomorphine in intrastriatally MPP^+-infused rats. On the contrary, in the present study, all the animals infused with MPP^+ or rotenone in striatum exhibited significant ipsilateral rotations following the DA-ergic drugs. The elicitation of contralateral rotations after apomorphine treatment in MFB-infused rats was expected as the lesion was "complete" (>90% striatal DA depletion) and the dwindling supply of DA to the DA receptors favored the establishment of DA receptor supersensitivity, as shown in intranigrally 6-OHDA-lesioned animals (Hefti et al., 1980).

It was surprising that intranigrally MPP^+-infused animals with a pronounced 75% depletion of DA in the striatum failed to evoke any contralateral bias in response to apomorphine. In the past, intranigrally MPP^+-infused animals have been reported to exhibit ipsilateral rotations following apomorphine (0.6 mg/kg, i.p.) challenge (Sun et al., 1988; Kondo et al., 2004). However, there are reports showing MPP^+-induced contraversive rotations in response to apomorphine when the neurotoxin is

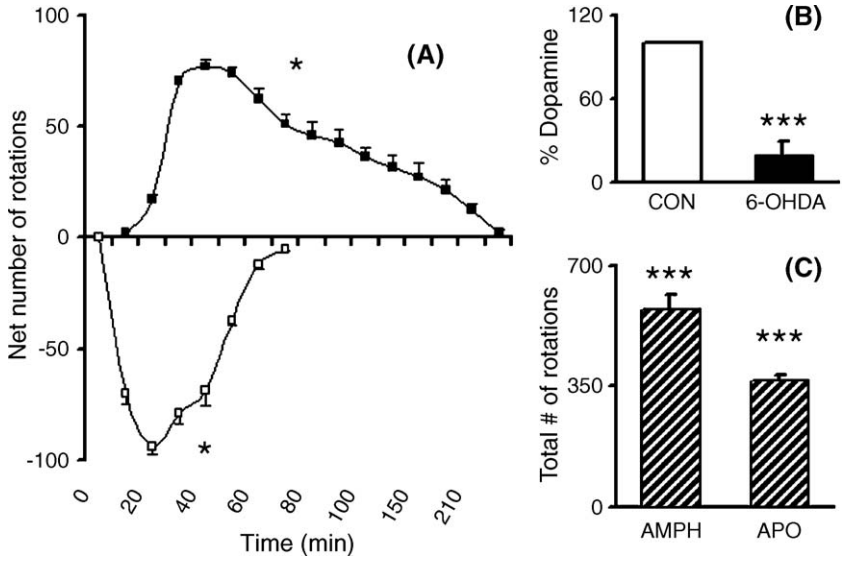

Fig. 4. Rotational response to DA-ergic drugs and changes in striatal levels of dopamine after 6-OHDA infusion. Animals were unilaterally infused with 6-OHDA (8 μg in 1 μl) in the substantia nigra pars compacta (SNpc) region. (A) Rats were tested for amphetamine induced rotations on 14th day and apomorphine response on 16th day post-infusion. Ipsilateral turnings are depicted as positive values and contralateral turnings as negative values. (B) Percentage DA level in the striatum of 6-OHDA infused rats, which were sacrificed 2 days after the last rotational study. (C) Total number of rotations elicited by 6-OHDA group for *d*-amphetamine and apomorphine administration. Data represented as mean±S.E.M. ($n=6$). *$p \leq 0.05$; ***$p \leq 0.001$ significantly different as compared to control animals.

infused intranigrally (Lange, 1989, 1990). It is interesting that, in the higher dose rotenone treated (12 μg in 1 μl/animal) animal model too, apomorphine elicited only ipsilateral rotations, despite more than 80% striatal DA depletion (Sindhu et al., 2005). It is possible that the level and duration of the striatal DA depletion in the ipsilateral striatum was not sufficient enough to create supersensitivity in the post-synaptic DA receptors. It has been reported that the spared DA-ergic neurons or availability of endogenous DA might interfere with the development of supersensitivity (Agid et al., 1973; Castaneda et al., 1990). Another possibility is the physical damage to post-synaptic membrane causing the post-synaptic DA receptors to be non-functional. However, in the absence of any experimental evidence, this aspect remains speculative.

One of the explanations for the above observation could be the dose of apomorphine administered in our study, which is comparatively high for detecting post-synaptic dopamine receptor supersensitivity. This may indicate that the large agonist effect may be producing erroneous rotational behavior as a consequence of the dose rather than the route of administration of rotenone or MPP^+. Since rotenone-infused animals did not show any rotational response with low dose of apomorphine (0.5 mg/kg, s.c.; see Sindhu et al., 2005), we were forced to administer similar doses in all our experimental animals for comparative purpose. This was warranted since the duration and number of rotations increased with increase in apomorphine dose (Ungerstedt, 1971; Iwamoto et al., 1976; Silverman and Ho, 1981).

The basal ganglia circuitry is complex, and GABA-ergic neostriatal efferents projecting mainly to SN pars reticulata (SNpr) and globus pallidus mediate the striatal DA-ergic activity. Ventral SNpr sends axons to thalamic nuclei, tectum, raphe nuclei, locus coeruleus, amygdala and septum (Clavier et al., 1976; Faull and Mehler, 1978; Nauta and Domesick, 1984), while it gets DA-ergic dendrites from SNpc. It could be possible that a lipophilic molecule like MPP^+ or rotenone could diffuse towards the ventral part of the nuclei of A_9 SN region, the SNpr that contain mainly D1 receptors (Beckstead et al., 1988; Besson et al., 1988). Loss of interaction of apomorphine to these extrastriatal DA receptors may be the cause of ipsilateral rotations obtained in our study. This will result in the damage of striatonigral outflow and inhibit the nigrofugal GABA-ergic neurons (Jasso-Lopez and Tapia, 1995) of SNpr, with projections mainly to the ventrolateral thalamic nucleus and the superior colliculus (Faull and Mehler, 1978; Deniau and Chevalier, 1985, 1992). It has been demonstrated that combined lesions in globus pallidus and SNpr elicit ipsilateral turnings to apomorphine administration (Konitsiotis et al., 1998). Therefore, it is possible that intranigrally MPP^+- or rotenone-infused animals have lesions extending to SNpr regions, influencing the net behavioral manifestations to DA receptor stimulation, whereas 6-OHDA being the specific DA-ergic toxin limited its action to SNpc area. We have observed rotenone lesions extending to SNpr in Nissl stained sections (Sindhu et al., 2005). In MFB-lesioned animals, both rotenone and MPP^+ elicited contralateral turnings to apomorphine, which is comparable to the 6-OHDA group. Thus, it could be concluded that MFB provides a better lesion site for modeling PD in regard to post synaptic drug receptor sensitivity screening.

We conclude that both rotenone and MPP^+ intranigrally can induce parkinsonian symptoms and can be used to produce experimental hemi-parkinsonism. The effects shown by rotenone are comparable with the one caused by MPP^+, which is a well-established parkinsonian neurotoxin. Furthermore, moderate striatal DA depletion in these models with intranigral infusion provides a valuable window for experimental therapeutic interventions. The present study provided several

attractive features of the MPP^+- and rotenone-infused animals as a model of PD. These observations further indicate that both MPP^+ and rotenone acted in a similar manner and elicited same kind of behavioral abnormalities. These results further suggest that, out of the three different lesion sites, MFB is the most sensitive region in the nigrostriatal axis to either MPP^+ or rotenone, since infusion of either of these neurotoxins into MFB in rats culminated in a maximum possible loss of the striatal DA and evoked contralateral rotations in response to apomorphine reflecting successful generation of supersensitivity of post-synaptic DA receptor in these models. Therefore, apomorphine induced contralateral rotations are a reliable indicator of specific destruction to the nigrostriatal pathway and development of post-synaptic dopamine receptor supersensitivity.

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