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5-HT_{2A} receptor antagonists improve motor impairments in the MPTP mouse model of Parkinson's disease

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Abstract

Clinical observations have suggested that ritanserin, a 5-HT_{2A/C} receptor antagonist may reduce motor deficits in persons with Parkinson's Disease (PD). To better understand the potential antiparkinsonian actions of ritanserin, we compared the effects of ritanserin with the selective 5-HT_{2A} receptor antagonist M100907 and the selective 5-HT_{2C} receptor antagonist SB 206553 on motor impairments in mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP-treated mice exhibited decreased performance on the beam-walking apparatus. These motor deficits were reversed by acute treatment with L-3,4-dihydroxyphenylalanine (levodopa). Both the mixed 5-HT_{2A/C} antagonist ritanserin and the selective 5-HT_{2A} antagonist M100907 improved motor performance on the beam-walking apparatus. In contrast, SB 206553 was ineffective in improving the motor deficits in MPTP-treated mice. These data suggest that 5-HT_{2A} receptor antagonists may represent a novel approach to ameliorate motor symptoms of Parkinson's disease.

Keywords

M100907; motor deficits; MPTP; ritanserin; SB 206553; serotonin

Parkinson's disease (PD) is a neurodegenerative disorder characterized by motor impairments, including rigidity, tremor, bradykinesia, and postural instability. These motor symptoms have been primarily attributed to the progressive degeneration of dopamine (DA) neurons in the substantia nigra and a concomitant loss of striatal DA (Lang and Luzano, 1998; Dauer and Przedborski, 2003; Fahn, 2003; Nutt and Wooten, 2005).

The motor symptoms of PD are treated by dopamine replacement therapy (DRT) with administration of either L-3,4-dihydroxyphenylalanine (levodopa) or direct dopamine agonists. Unfortunately, most patients so treated develop motor fluctuations and abnormal involuntary movements (dyskinesias) after several years (Lang and Luzano, 1998; Obeso et al., 2000; Dauer and Przedborski, 2003; Fahn, 2003; Nutt and Wooten, 2005). There is a clear need to identify non-dopaminergic drug targets to provide fewer side effects while maintaining therapeutic efficacy.

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Clinical studies have suggested that serotonin-2 (5-HT₂) receptor antagonists may be useful in the treatment of the motor symptoms of PD. For example, ritanserin, a mixed 5-HT_{2A/C} receptor antagonist, has been shown to reduce bradykinesia and improve gait in PD patients (Henderson et al., 1992), as well as ameliorate neuroleptic-induced parkinsonism (Bersani et al., 1990). Lucas and coworkers (1997) showed that the reduction of neuroleptic-induced catalepsy in rats by ritanserin was independent of changes in DA dynamics. These actions of ritanserin may be related to the localization of striatal 5-HT_{2A} receptors to corticostriatal and pallido-striatal neurons (Bubser et al., 2001), where they serve as heteroreceptors to regulate glutamate release (Scruggs et al., 2003).

To explore the role of 5-HT₂ receptors on motor dysfunction in parkinsonism, we used the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of parkinsonism in mice. MPTP treatment of susceptible species results in a marked depletion of striatal DA stores and loss of dopamine neurons in the substantia nigra (Heikkila et al., 1984; Sundstrom et al., 1987; Stephenson et al., 2007). We assessed motor function of MPTP-treated mice using the open field test, rotarod apparatus, and the beam traversing task (Fernagut et al., 2004; Allbutt and Henderson, 2007; Quinn et al., 2007). We then compared the effects of mixed and subtype selective 5-HT₂ receptor antagonists with the indirect dopamine agonist levodopa on MPTP-induced motor deficits.

Materials and Methods

Animals

Male C57BL/6J mice, 70-77 days of age at the start of experiments, were obtained from Jackson Labs (Bar Harbor, ME). Animals were group housed in a temperature and humidity controlled room and maintained on a 12L:12D light-dark cycle (lights off at 1900h). Food and water were provided *ad libitum*. All studies were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and under the oversight of the Meharry Medical College Animal Care and Use Committee.

Drug treatments

Mice were injected with 20 mg/kg (ip) MPTP (Sigma-Aldrich, St. Louis, MO) or saline every two hours for a total of four injections, resulting in a cumulative dose of 80 mg/kg; there was a mortality rate of 5% using this treatment protocol.

All behavioral tests were done three weeks after MPTP administration. Where indicated, animals were subjected to motor function tests 20 minutes after intraperitoneal injection of 15 mg/kg L-DOPA methyl ester (Sigma-Aldrich) with 12.5 mg/kg benserazide (Sigma-Aldrich), a peripheral dopa decarboxylase inhibitor. Serotonergic antagonists were injected intraperitoneally 30 min before behavioral testing. These included ritanserin (Sigma-Aldrich) and SB 206553 (Sigma-Aldrich), which were dissolved in saline containing 0.4% acetic acid and adjusted to pH 6.5-7.0 with NaOH (Leslie et al., 1993), and the selective 5-HT_{2A} receptor antagonist, M100907 [(R-(+)-alpha-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenethyl)]-4-piperidinemethanol]; Kehne et al., 1996), which was dissolved in 0.1 M tartaric acid solution which adjusted to pH 6.5-7.0 with NaOH. All injection volumes were 10 ml/kg. All mice received a single dose of either vehicle or an active pharmacological agent; animals did not receive different doses of the various drugs examined. Thus, all comparisons were between subjects. We chose a broad range of doses encompassing effective doses that can block the specific receptor. The doses of drugs used were based on the available literature (Leslie et al., 1993; Kehne et al., 1996; De Deurwaerdere et al., 2004) as well as pilot studies which showed that the selected doses did not alter general locomotor behavior.

Open Field Test

Each mouse was placed in an activity monitor measuring 27.3 cm × 27.3 cm (Med Associates, St. Albans VT) and locomotor activity and rearing (measured as interruptions of photobeams at different heights in the cage) were recorded in 10 min bins for 60 min.

Rotarod Test

Each mouse was placed in a separate lane of a rotarod (Med Associates, St. Albans VT) on a 5-cm diameter rotating cylinder at 5 rpm. The mice were first placed on the rubber covered cylinder and left for 30 s to habituate to the rod without rotation. The rotarod was started and brought slowly up to the required speed of 5 rpm. The performance was recorded as the mean latency to fall from the rod (up to 300 s) in four consecutive trials with a 5-min resting period between each trial.

Beam Walking Apparatus

The apparatus used in this experiment was a modification of that used by Allbutt and Henderson (2007). It consisted of a 1 cm square stainless steel beam of 105 cm in length. The beam was suspended 49 cm above the floor of the test chamber. Mice were habituated to the goal box for 3 minutes, then placed a distance of 10 cm from goal box. They were allowed to traverse the beam to the goal box. Upon successful traversal of the beam to the goal box at the 10 cm distance, mice were placed at increasing distances of 30, 50, and 80 cm from the goal box and trained to traverse the beam for one trial at each distance for two consecutive days. Mice able to traverse the full 80 cm length of the beam to the goal box within 60 seconds were considered to reach criterion. Three weeks post-MPTP treatment mice were subjected to three consecutive trials on the beam during which time they were videotaped. The number of footslips off the beam in each trial, and the mean number of hindlimb footslips during a three-trial session, were recorded (Dluzen et al., 2001; Fernagut et al., 2004; Strome et al., 2006; Allbutt and Henderson 2007; Quinn et al., 2007; Urakawa et al., 2007) by persons unaware of the treatment condition of the animals.

Regional monoamine concentrations

Separate groups of untreated animals were used to determine the effects of MPTP treatment on regional monoamine concentrations. Animals were sacrificed three weeks after saline or MPTP administration. This is the same time point that the behavioral studies were performed. These animals did not receive pharmacological agents. The tissues harvested for monoamine determination included the dorsal precommissural striatum, nucleus accumbens (including both core and shell), substantia nigra, and cerebellum. The tissues were dissected from 1.0 mm thick coronal slices (Deutch et al., 1985) and samples stored at -80° C until assayed. Regional concentrations of dopamine, serotonin, and their acidic metabolites were determined by HPLC-EC as previously described (Deutch and Cameron, 1992), with protein concentrations determined by the method of Lowry et al. (1951).

Statistical Analysis

Data on monoamine concentrations (ng amine/mg protein) in MPTP-treated and control animals were analyzed by Student's t-test; alpha levels (corrected for multiple comparisons) were set at 0.05. Two-way ANOVAs were used to analyze the behavioral effects of levodopa, ritanserin, M100907 and SB 206553, with *post hoc* tests when indicated by a significant main effect or interaction. Because baseline footslips did not vary across different experiments, the footslip data from the control groups were pooled.

Results

Behavioral Studies

MPTP treatment did not result in a significant difference in locomotor activity, as measured in the open field, nor did it significantly impair rotarod performance (see Figure 1B and 1C). In contrast, a two-way ANOVA revealed a significant main effect of MPTP treatment on the number of footslips as the mice traversed the beam in the beam walking task (overall $F_{3,81} = 40.88$, $p < 0.001$; MPTP treatment effect $F_{1,81} = 29.86$, $p < 0.001$; see Figure 1A). Post hoc tests demonstrated MPTP treatment caused a marked increase in the number of footslips ($p < 0.001$). The impaired performance on the beam walking task was completely reversed by levodopa (overall $F_{3,81} = 40.88$, $p < 0.001$; drug (levodopa) main effect $F_{1,81} = 14.81$, $p < 0.001$; treatment $\times$ drug interaction $F_{1,81} = 28.43$, $p < 0.001$; see Figure 1A).

Ritanserin also resulted in a dose-dependent decrease in the number of footslips as the mice traversed the beam (see Figure 2), with significant main effects on the MPTP treatment ($F_{1,135} = 68.67$, $p < 0.001$) and drug ($F_{3,135} = 11.50$, $p < 0.001$) conditions, as well as a significant MPTP condition $\times$ drug interaction ($F_{3,135} = 12.05$, $p < 0.001$). However, despite the fact that a significant improvement in performance was noted even at the lowest dose of ritanserin tested (0.01 mg/kg), at no dose did ritanserin completely reverse the performance deficit in beam walking (see Figure 2).

Results similar to those seen with ritanserin were obtained in testing the effects of M100907. Thus, ANOVA revealed significant MPTP treatment ($F_{1,139} = 70.47$, $p < 0.001$) and drug ($F_{3,135} = 11.23$, $p < 0.001$) main effects, as well as a significant interaction ($F_{3,135} = 11.72$, $p < 0.001$) (see Figure 3). Significant main effects were seen with the lowest dose tested (0.1 μ g/kg), with no significant difference seen between the 1.0 and 10.0 μ g/kg doses (see Figure 3). As was the case with ritanserin, M100907 did not completely reverse the MPTP-induced performance deficit (see Figure 3).

In contrast to the effects of ritanserin and M100907, the 5-HT_{2C} antagonist SB 206553 did not significantly improve performance on the beam task (see Figure 4). Thus, although there was a significant MPTP treatment effect ($F_{1,146} = 253.34$, $p < 0.001$), no significant drug main effect was uncovered, nor was there a significant MPTP $\times$ drug interaction ($F_{3,146} = 1.275$; NS).

Neurochemical Assessment

MPTP treatment decreased striatal dopamine (DA) concentrations by 79% relative to saline-treated controls (see Table 1). Concentrations of the DA metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) were also significantly decreased, and consistent with a large but incomplete lesion of the striatal DA innervations. DA utilization (DOPAC/DA) was significantly increased (see Table 1). DA concentrations were also significantly lower in the nucleus accumbens and substantia nigra than in saline-injected control mice (Table 1), but no difference in cerebellar DA levels was uncovered. In contrast to the significant decreases in nigrostriatal DA levels, concentrations of serotonin did not differ significantly between MPTP- and saline-treated mice, nor were any differences in serotonin utilization (5-HIAA/5-HT) uncovered. Although MPTP treatment did not alter 5-HT levels in the striatum and nucleus accumbens, a significant decrease in 5-HT concentration and that of its metabolite 5-hydroxyindolacetic acid (5-HIAA) was seen in the substantia nigra (Table 1).

Discussion

Levodopa is arguably the most effective treatment for PD, but patients invariably develop motor fluctuations and dyskinesias with chronic treatment (Lang and Luzano, 1998; Obeso et

al., 2000; Dauer and Przedborski, 2003; Fahn, 2003; Nutt and Wooten, 2005). Treatment with direct DA agonists also causes dyskinesias, although there may be a longer interval between initiation of treatment and emergence of dyskinesias with agonists. Thus, although dopamine replacement therapy is remarkably effective in the treatment of idiopathic PD, the side effect liabilities of dopaminergic agents suggest that drugs that modulate the activity of striatal medium spiny neurons may be a useful strategy to improve motor function, perhaps with lower side effect liability. The limitations associated with dopaminergic agents prompted us to investigate the potential antiparkinsonian actions of 5-HT₂ receptor antagonists.

We failed to observe a significant effect of MPTP treatment on locomotor activity, as assessed in the open field apparatus, or any significant decrement in performance in the acute rotarod task. In contrast, we observed a marked and reproducible decrease in performance on the beam traversing task. Previous examinations of the effects of MPTP treatment on locomotor activity have yielded mixed results, with some reports noting a decrease (Arai et al., 1990; Frederickson et al., 1990; Luchtman et al., 2009) and others an increase (Chia et al., 1996; Rousselet et al., 2003), but most reporting no significant change in activity (Nishi K. et al., 1991; Tomac et al., 1995; Tillerson et al., 2002; Mori et al., 2005). Similarly, studies of performance on the acute rotarod test have yielded inconsistent results, with different studies reporting both decreases (Rozas et al., 1998; Luchtman et al., 2009) and no change in MPTP-treated mice relative to control subjects (Sedelis et al., 2000; Tillerson et al. 2002). Some of the variance across reports probably reflects technical difference in the task apparatus or testing, or differences in interval between MPTP treatment and testing or in the MPTP treatment protocol used. The latter factors have a large influence on the extent of the degree of striatal dopamine loss, and as importantly the residual striatal dopamine (Bezard and Gross, 1998).

We did observe that mice treated with MPTP were significantly impaired on the beam walking apparatus. Quinn et al. (2007) similarly reported that MPTP decreases performance in this task. In addition, impairments in beam walking that were significantly attenuated by levodopa have been reported in the aphakia (pit3x-deficient) transgenic mouse model of parkinsonism (Hwang et al., 2005).

We found that levodopa, at doses previously shown to reduce other motor deficits in animal models of parkinsonism (Fredricsson et al., 1990; Tillerson et al., 2003), reversed completely the number of footslips in the beam walking task. This finding, taken together with earlier data, suggests that performance on the beam walking test has some predictive validity for PD.

Ritanserin dose-dependently attenuated the number of footslips in the beam walking test, although at none of the doses tested was there a complete reversal of the motor deficit. The selective 5-HT_{2A} antagonist M100907 in a dose related manner decreased the number of footslips, having a significant effect even at the lowest dose (0.1 µg/kg); however, we did not observe a complete reversal of footslips to control levels by a higher dose of M100907. In contrast to the beneficial effects of ritanserin and M100907, the 5-HT_{2C}-preferring antagonist had no effect on performance of MPTP-treated mice in the beam walking test. These data suggest that the ability of the 5-HT_{2A/C} antagonist ritanserin to reduce parkinsonian motor deficits is attributable to actions at the 5-HT_{2A} but not 5-HT_{2C} receptor.

Consistent with previous studies, MPTP treatment markedly decreased striatal DA concentrations, with a lesser but significant effect in the ventral striatum (nucleus accumbens; Heikkila et al., 1984; Sundstrom et al., 1987; Stephenson et al., 2007; Nayyar et al., 2009). In contrast, we did not observe a change in striatal 5-HT concentrations. This finding is consistent with many but not all previous reports in the rodent (Hara et al., 1987; Sundstrom et al., 1987; Rozas et al., 1998; Rousselet et al., 2003; Stephenson et al., 2007; Vuckovic et al., 2008). The lack of consistent changes in nigrostriatal serotonin concentrations in the MPTP-

treated mouse probably reflects the use of different MPTP treatment protocols and post-treatment survival periods (see Nayyar et al., 2009).

There have been conflicting results concerning the effects of striatal dopamine depletion on 5-HT_{2A} receptors in the striatum. Zhang et al. (2007) noted an increase in striatal levels of the 5-HT_{2A} transcript in response to 6-hydroxydopamine (6-OHDA) lesion-induced dopamine denervation. In contrast, Li et al. (2010) reported that the density of 5-HT_{2A} receptors, as measured by assessing [3H]ketanserin binding sites, was decreased by 6-OHDA lesions of the median forebrain bundle. We are not aware of any published imaging studies of striatal 5-HT_{2A} binding potential in idiopathic PD.

We found that ritanserin improved motor function in MPTP-treated mice. This observation is consistent with clinical data showing that ritanserin reduces bradykinesia and improves gait in PD patients (Henderson et al., 1992), as well as reduces antipsychotic drug-induced extrapyramidal side effects (Bersani et al., 1990; Miller et al., 1990, 1992). Because SB206553 failed to improve MPTP-induced motor deficits, the anti-parkinsonian action of ritanserin is probably not due to 5-HT_{2C} receptor blockade. There are conflicting views on the role of 5-HT_{2C} receptors in the function of the nigrostriatal DA system. Some studies indicate that 5-HT_{2C} receptors exert tonic inhibitory control on the nigrostriatal system (De Deurwaerdere and Spampinato, 2001; De Deurwaerdere et al., 2004), whereas other reports have found no effect (Di Matteo et al., 2001).

Our results are in agreement with a recent study showing that the 5-HT_{2A} receptor inverse agonist, ACP-103 (pimavanserin) reduced parkinsonian tremor in rats (Vanover et al., 2008). Whereas our studies have investigated the anti-parkinsonian action of 5-HT_{2A} receptor antagonists other studies have documented the involvement of 5-HT_{2A} receptors in levodopa-induced dyskinesia (LID). M100907 reduced dyskinesia induced by the dopamine D1 agonist SKF 82958 but not that induced by levodopa (Taylor et al., 2006). SB 206553 has been shown to enhance the antiparkinsonian effects of dopamine D1 and D2 agonists in 6-hydroxydopamine-lesioned rats with no effects on rotational behavior when administered alone (Fox and Brotchie, 2000).

The very high density of striatal 5-HT_{2A} binding sites stands in contrast to the very low abundance of 5-HT_{2A} mRNA in striatal medium spiny neurons (MSNs) (Pompeiano et al., 1994; Ward and Dorsa, 1996; Mijster et al., 1997; Bubser et al., 2001). This is consistent with the finding that the majority of 5-HT_{2A} receptors is localized to the terminals of corticostriatal axons (Bubser et al., 2001). The loss of striatal dopamine signaling through D₂ receptors removes a tonic inhibitory constraint on cortico-striatal glutamatergic axons (DeLong, 1990; Bamford et al., 2004). Anatomical studies of the dopamine-denervated striatum have revealed an increase in the density of perforated synapses onto MSNs dendritic spines, suggestive of increased excitatory (glutamatergic) drive onto striatal projection neurons (Anglade et al., 1996; Meshul et al., 1999, 2002; Raju et al., 2008). Consistent with the anatomical studies, *in vivo* microdialysis studies and proton magnetic resonance spectroscopy findings have suggested increased glutamate concentrations in the striatum of MPTP-treated mice (Robinson et al., 2003; Chassain et al., 2008).

Several studies suggest that normalizing increased glutamatergic drive over MSNs would be of therapeutic benefit in PD (Starr, 1995; 1998; Blandini et al., 1996; Jankovic and Hunter, 2002; Garcia et al., 2010), but clinical trials have failed, mainly because of side effects. We hypothesize that 5-HT_{2A} heteroreceptors present on corticostriatal axons dampen glutamate release and thereby may be of use in treating the motor deficits of PD. Activation of 5-HT_{2A} heteroreceptors in several brain areas has been shown to evoke glutamate release (Aghajanian and Marek, 1997; Scruggs et al., 2003).

In dopamine-depleted rats 5-HT_{2A} receptors mediate effects associated D₁ receptor function. It has been reported that treatment with D₁ and 5-HT₂ agonists produce synergistic induction of striatonigral gene expression (Gresch and Walker, 1999) and hyperlocomotion (Bishop and Walker, 2003). The hyperlocomotion response was suppressed by the selective 5-HT_{2A} receptor antagonist M100907 (Bishop et al., 2005). Thus it appears that in the dopamine-depleted brain, 5-HT_{2A} receptors may regulate the direct striatal output pathway as well as the indirect glutamatergic pathway. However, because the great majority of striatal 5-HT_{2A} receptors are localized to corticostriatal terminals (Bubser et al., 2001), a more plausible mechanism to explain our findings is an indirect action occurring through regulation of glutamate release. Future studies designed to test the relative contributions of direct vs indirect effects on MSN function will be required to define which mechanism is operative.

In conclusion, we find that 5-HT_{2A} receptor antagonism improves motor impairments in MPTP-treated mice. This finding is consistent with earlier studies suggesting an antiparkinsonian effect of ritanserin, and specifically point to 5-HT_{2A} receptors as providing a novel target for the treatment of parkinsonian symptoms. Further clinical exploration of 5-HT_{2A} receptor antagonists as potential therapeutic agents in PD is warranted.

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Figure 1.

Motor performance in MPTP-treated mice. Data are presented as mean $\pm$ SEM. (A) MPTP treatment produced significant increase in the number of footslips. The number of footslips in MPTP-treated mice was decreased by L-DOPA when compared with saline-treated mice. Because baseline footslips did not vary across different experiments, the footslip data from the vehicle groups were pooled (Vehicle: $n = 31$ /group; L-DOPA: $n = 10$ /group). (B) MPTP treatment did not alter locomotor activity of MPTP-treated mice ($n = 12$) in the open field test when compared to saline-treated mice ($n = 8$). (C) Motor performance on the rotarod ($n = 10$ /group). *** significantly different from saline-treated mice ($P < 0.001$); ### significantly different from MPTP-treated mice ($P < 0.001$), in two-way ANOVA with Tukey's post hoc comparison.

Figure 2.

Ritanserin decreased the number of footslips in MPTP-treated mice. Data are presented as mean $\pm$ SEM. Ritanserin produced dose-dependent attenuation of motor impairments exhibited by MPTP-treated mice on the beam-walking apparatus but had no effect on the performance of saline-treated mice. The footslip data from the vehicle groups were pooled (Vehicle: $n = 31$ /group; Ritanserin: $n = 12$ /group). *** significantly different from saline-treated mice ($P < 0.001$); ## significantly different from MPTP-treated mice ($P < 0.01$); ### significantly different from MPTP-treated mice ($P < 0.001$); in two-way ANOVA with Tukey's post hoc comparison.

Figure 3.

M100907 improved performance of MPTP-treated mice on the beam walking apparatus. Data are presented as mean $\pm$ SEM. M100907 dose-dependently reduced the number of footslips in MPTP-treated mice but had no effect on saline-treated mice. The footslip data from the vehicle groups were pooled (Vehicle: $n = 31$ /group; M100907: $n = 13$ /group). *** significantly different from saline-treated mice ($P < 0.001$); ### significantly different from MPTP-treated mice ($P < 0.001$), in two-way ANOVA with Tukey's post hoc comparison.

Figure 4.

SB 206553 failed to improve motor deficits in MPTP-treated mice. Data are presented as mean $\pm$ SEM. SB 206553 had no effect on footslips of both groups of mice ($n = 10/\text{group}$). The footslip data from the vehicle groups were pooled (Vehicle: $n = 31/\text{group}$; SB 206553: $n = 13/\text{group}$). *** significantly different from saline-treated mice ($P < 0.001$) in two-way ANOVA with Tukey's post hoc comparison.

Table 1

Levels of DA, 5-HT, and their metabolites, DOPAC and 5-HIAA measured by HPLC in brain regions of saline and MPTP-treated mice.

	Treatment	DA	DOPAC	DA Utilization
Striatum	Saline	64.91±3.53	4.65±0.34	0.07±0.004
	MPTP	13.66±1.37**	1.37±0.12**	0.10±0.006*
S. Nigra	Saline	3.39±0.36	1.05±0.07	0.32±0.01
	MPTP	2.14±0.36*	0.67±0.09*	0.34±0.03
N. Accumbens	Saline	14.07±0.54	2.36±0.16	0.17±0.01
	MPTP	8.71±0.67**	1.42±0.08**	0.17±0.01
Cerebellum	Saline	0.07±0.01	0.05±0.02	0.79±0.29
	MPTP	0.05±0.03	0.01±0.01	1.63±0.84
		5-HT	5-HIAA	5-HT Utilization
Striatum	Saline	1.07±0.14	0.59±0.02	0.63±0.08
	MPTP	0.98±0.07	0.56±0.02	0.59±0.05
S. Nigra	Saline	11.21±0.53	6.56±0.22	0.60±0.04
	MPTP	9.05±0.74*	4.87±0.45*	0.54±0.04
N. Accumbens	Saline	1.19±0.07	1.80±0.23	1.51±0.18
	MPTP	0.92±0.05	1.09±0.09	1.22±0.11
Cerebellum	Saline	0.97±0.18	2.81±0.12	3.65±0.61
	MPTP	0.72±0.05	2.44±0.21	3.39±0.13

Data are presented as mean ± SEM of monoamine concentrations (in ng/mg protein).

Dopamine utilization was calculated as [DOPAC]/[DA] and serotonin utilization was calculated as [5-HIAA]/[5-HT].

* p<0.05;

** p<0.001; –significantly different from saline group (N=8/group).