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Amphetamine Induces Hypermotility in MPTP-Lesioned Mice

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SCHROEDER, U., M. R. KREUTZ, H. SCHROEDER AND B. A. SABEL. *Amphetamine induces hypermotility in MPTP-lesioned mice.* PHARMACOL BIOCHEM BEHAV. **56**(2) 281–285, 1997.— Two strains of mice (NMRI and C57Bl/6) were treated with MPTP (within 8 h 4 × 30 mg/kg MPTP, IP) and motility was monitored 10 days later. An acute administration of amphetamine (2.5 mg/kg or 10.0 mg/kg) or apomorphine (0.5 mg/kg or 5.0 mg/kg) led to hypermotility and a dose-dependent increase of stereotyped behavior. Immunocytochemical investigations indicated a substantial loss of tyrosine-hydroxylase immunoreactivity in the basal ganglia which was accompanied by a 15% increase of ^{3}H-spiroperidol binding to a striatal membrane preparation. No difference was found in biochemical and behavioral measures between both mice strains. Thus, MPTP-induced lesions in mice are probably followed by a denervation-like supersensitivity of the dopaminergic system, which might account for the finding that despite a severe degeneration of dopaminergic terminals amphetamine induces hypermotility. **Copyright © 1997 Elsevier Science Inc.**

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) Parkinson disease Mouse Dopamine Locomotor activity Spiroperidol binding Supersensitivity

SYSTEMIC administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is accompanied by the development of Parkinsonian-like symptoms in primates, and to a lesser extent, in rodents (9,20). These symptoms are the result of the loss of neurons in the zona compacta of the substantia nigra with a corresponding decreased of dopamine levels in the striatum (5). The mechanisms of MPTP action are still not fully understood. It has been proposed that MPTP acts via (i) an inhibition of the complex I in the mitochondrial respiratory chain by its major metabolite N-methyl-4-phenylpyridine (MPP+); (ii) a formation of free radicals or peroxides and (iii) disturbances of intracellular calcium homeostasis (4,17).

In various mice strains MPTP administration is associated with a loss of cells in the pars compacta of the substantia nigra and severe reductions in the concentrations of dopamine, noradrenaline and serotonin in the striatum (4). Many studies have examined motor aspects of Parkinsonian-like syndromes in MPTP-treated monkeys (3,18). In mice, however, MPTP-induced motor deficits are less consistent and rather variable, depending upon the mice strain used and the dose employed. Acute MPTP treatment produces a transient behavioral syndrome which includes tremor, jumping, akinesia and others (7,14), but chronic behavioral alterations were not yet observed. Also not much is known about drug-induced motor behavior in MPTP-lesioned mice. In order to gain a better understanding of the psychopharmacology of the partially denervated striatum it would be useful to characterize behavioral alterations in mice which are elicited by dopaminergic drugs. We have therefore investigated the effects of amphetamine and apomorphine on stereotyped and locomotor behavior in two MPTP treated mice strains and studied cell loss and dopamine D_2 receptor binding as well.

METHODS

Animals

Seventy seven male mice (22-25 g b.wt., Tierzucht Schönwalde; Germany) from the strains NMRI and C57Bl/6 were used. The animals were housed in plastic cages, food and water were available ad libitum. Animals were maintained in temparature- and humidity-controlled rooms with a 12 h light–dark cycle.

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MPTP-Induced Lesions

Animals were injected intraperitoneally with 30 mg/kg MPTP-hydrochloride (Biotrend, Germany), 4 times within 8 h. Control animals received 4 injections with isotonic saline.

Measurement of Locomotor Activity

On post-injection day 10, a single test dose of D(+)-amphetamine (Sigma, Germany; 2.5 or 10.0 mg/kg b.wt. IP; $n = 8$ animals per group) or a single dose of apomorphine (Woelm Pharma, Germany; 0.5 or 5.0 mg/kg b.wt. IP; $n = 8$ animals per groups) were injected. After 20 min the locomotor activity was determined for another 20 min with an activity meter (Moti-Testsytem, TSE Bad Homburg, Germany). Total ambulation scores, crossed squares, and the duration and frequency of rearings were measured.

Stereotypy

Stereotyped behavior was rated every 5 min by an observer who was not aware of the animals' group identity during the measurement of the locomotor activity. Overall stereotypy was assessed by use of a 6-point scale described by Kelly and Iversen (8), in which scores were given as follows: 0 = inactive; 1 = normal activity, 2 = normal activity with short phases of stereotype sniffing and rearing, 3 = stereotype sniffing and rearing in whole room, 4 = stereotype sniffing and rearing in whole room with phases of head weaving at one point, 5 = stereotype sniffing, rearing and head weaving with phases of licking and biting at one point, 6 = continuous licking and biting.

Tyrosine Hydroxylase-Immunoreactivity

Deeply anesthetized animals were perfused with 10 ml of 0.1 M phosphate buffer saline (pH 7.4) followed by 10–20 ml of 4% paraformaldehyde in 0.1M PBS. Brains were removed, shock-frozen and frontal sections were cut on a cryostat (Model 2800 Frigocut, Leica Instruments, Germany) at 20 μm. Sections were then processed for tyrosine hydroxylase-immunochemistry. The tissue was postfixed with 4% paraformaldehyde for 5 min. After washing in 0.1 M Tris-buffer (pH 7.4), sections were exposed to 5% normal swine serum in 0.1 M PBS for 30 min and transferred to a 1 : 400 dilution of primary antibody (Eugene Tech., Ramsey, NJ, USA). Following overnight incubation at room temperature slides were rinsed and incubated with a biotinylated secondary antibody (Vectastain ABC) for 30 min, rinsed repeatedly and transferred to Vectastain ABC solution/phosphate buffer for 30 min. As a chromogen 3,3-diaminobenzidine (DAB; Sigma) was used at a concentration of 0.05% in 0.1 M phosphate buffer, supplemented with 0.01% H_2O_2 for 8 min. Sections were then dried and coverslipped with DePeX (Serva, Heidelberg, Germany).

^3H Spiroperidol Binding

Ten days after MPTP-induced lesions additional mice which were not treated with dopaminomimetics were decapi-

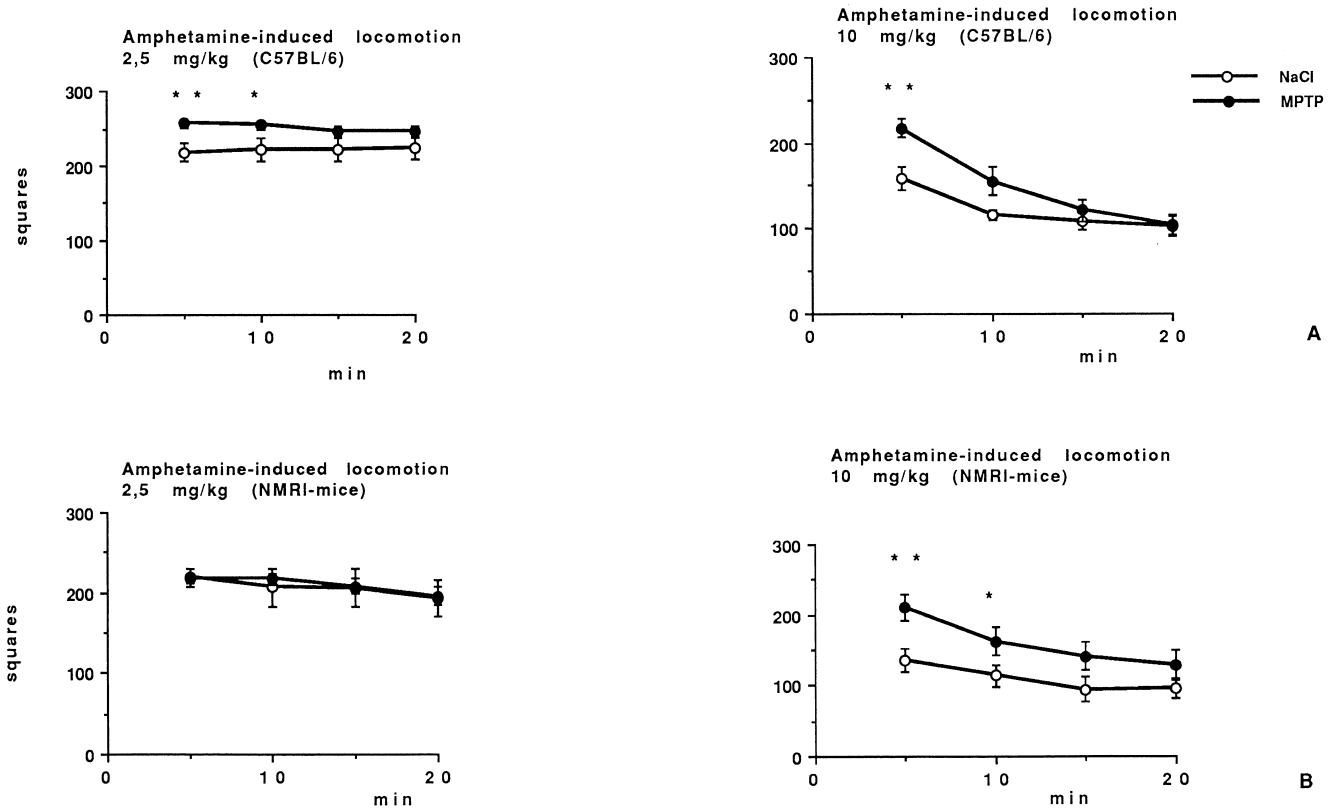

FIG. 1. The influence of amphetamine (2.5 mg/kg or 10 mg/kg) on locomotor acitivity of C57Bl/6 (A) and NMRI (B) mice after MPTP lesion as measured by number of crossed squares in an activity meter. (open circles = unlesioned group; black circles = MPTP-lesioned group; $n = 8$ for all groups); $^*p < 0.05$ compared to controls, mean ± SEM.

tated, their brain rapidly removed and striata dissected out. Crude membrane fractions were prepared by a modified method of Zukin (21). Briefly, to prepare a 5% homogenate (wet weight/volume) tissue was homogenized in 50 mM Tris-HCl buffer, pH 8.0, containing 120 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$ and 50 nM cianserin (to block 5-HT_2-receptors) and stored at −20°C. After thawing, homogenates were centrifuged for 10 min at 50,000 × g. The resulting pellet was washed with homogenization buffer and centrifuged again. ^{3}H-spiroperidol binding was measured using the method of Leysen (11). Specific binding was calculated by subtracting non-specific binding defined as that seen in the presence of 1 nM ^{3}H-spiroperidol plus 2 μM unlabelled *d*-butaclamol from total binding obtained with 1 nM ^{3}H-spiroperidol alone. The pellet was resuspended with Tris-HCl buffer. Aliquots of the crude membrane suspension containing 150–250 μg protein were mixed with 3H-spiroperidol (specific activity 800 GBq/mmol, Amersham, UK) and incubated for 30 min at 37°C. The reaction was terminated by rapid filtration under reduced pressure through GF/A glass-fiber filters. Filters were washed three times with buffer and taken for liquid scintillation counting in toluene-containing solvent. The protein content was determined according to the method described by Lowry (12).

Statistics

Statistical significance was assessed by analysis of variance (ANOVA) followed by appropriate post-hoc comparisons (Tukey-test).

RESULTS

Behavior

Injections of MPTP did not alter the spontaneous locomotor activity in both mice strains in comparison to saline treated controls (data not shown). The application of 2.5 mg/kg amphetamine, however, led to an increased ambulatory activity in MPTP-treated C57Bl/6 mice ($p < 0.05$) but not in NMRI mice. Hence, the higher amphetamine dose of 10 mg/kg further increased the motility in both strains ($p < 0.05$) compared to unlesioned animals (Fig. 1A and B).

Moreover, in the NMRI strain no difference in locomotor activity between lesioned and unlesioned animals were found after an acute treatment with 0.5 mg/kg apomorphine. The application of 5.0 mg/kg apomorphine, in contrast, resulted in a significant increase ($p < 0.05$) of locomotor activity of MPTP-lesioned animals (Fig. 2). The determined stereotypy scores showed some contradictary results. The lower amphetamine dose enhanced ($p < 0.05$) the stereotypy in the C57Bl/6 mice strain whereas the higher dose decreased ($p < 0.05$) the measured scores in the NMRI strain. After apomorphine treatment no difference was observed between C57Bl/6 and NMRI mice (Fig. 3).

Tyrosinehydroxylase-Immunoreactivity

As expected a clear reduction of tyrosinehydroxylase (TH) immunoreactivity was found in MPTP-treated animals. Especially in the basal ganglia the staining intensity was much lower than in controls, indicating a severe degeneration of dopaminergic terminals 10 days after MPTP treatment (Fig. 4). Also the number of TH-positive neurons and the intensity of TH-immunoreactivity in the substantia nigra was reduced. However, this reduction was much less intense than in the basal ganglia. No obvious difference was found between animals of both strains (data not shown).

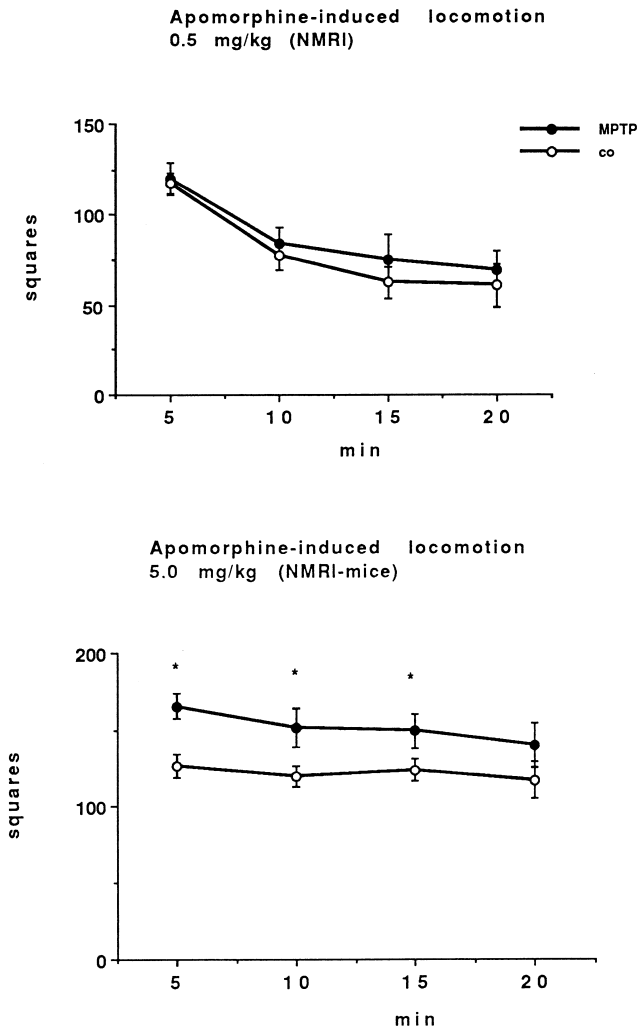

FIG. 2. The influence of apomorphine (0.5 mg/kg and 5.0 mg/kg) on locomotor activity of NMRI mice strain after MPTP lesion as measured by number of crossed squares in an activity meter. (open circles = unlesioned group; black circles = MPTP-lesioned group; $n = 8$ for all groups); *$p < 0.05$ compared to controls, mean ± SEM.

Biochemistry

The MPTP-induced lesion was accompanied by a 12% increase of striatal ^{3}H-spiroperidol binding to crude striatal synaptic membranes. Specific ligand binding was 546 ± 18 in untreated animals ($n = 6$) and 607 ± 11 for MPTP-lesioned ($n = 7$) animals (means fmol/mg protein ± SEM, $p < 0.05$).

DISCUSSION

MPTP-induced dopaminergic lesions in primates and rodents are associated with a Parkinsonian-like syndrom. In the present study two mice strains, NMRI and C57Bl/6, were treated with MPTP and motility/stereotypy scores were monitored 10 days later in response to an acute administration of apomorphine and amphetamine. No obvious effects were

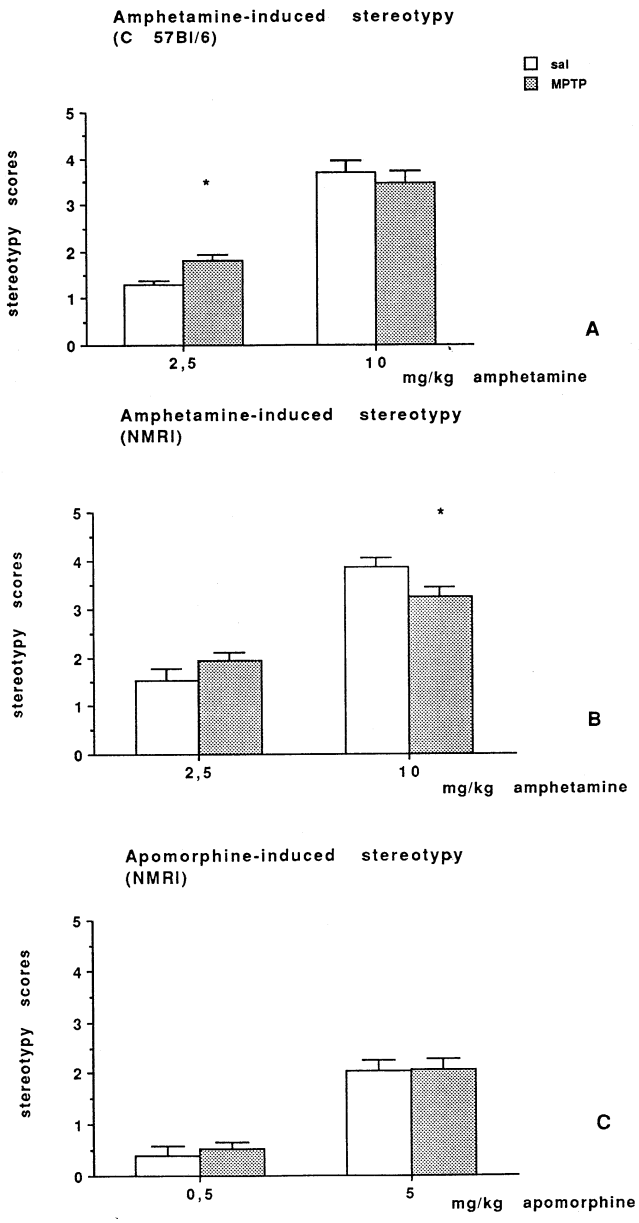

FIG. 3. The effect of amphetamine (2.5 mg/kg and 10 mg/kg) and apomorphine (0.5 mg/kg and 5.0 mg/kg) on stereotypy behavior of C57Bl/6 and NMRI mice after MPTP lesion. (open column = unlesioned group; black column = MPTP-lesioned group); *$p < 0.05$ compared to controls.

found in spontaneous locomotor behavior. The dose regimen of MPTP employed in this study has been shown previously to induce a severe depletion of striatal dopamine stores for several weeks (15,19). Surprisingly, we found that irrespective of the drastic nigrostriatal lesion amphetamine is still capable to induce hypermotility. Amphetamine increases locomotor behavior via a Ca^{2+}- independent increase of extracellular dopamine levels, which is mainly induced by a presynaptic dopamine reuptake block and a reversal of the Na+/K+ ATPase at the dopamine transporter with an accompanying efflux of cytosolic dopamine (2). Therefore we speculate that

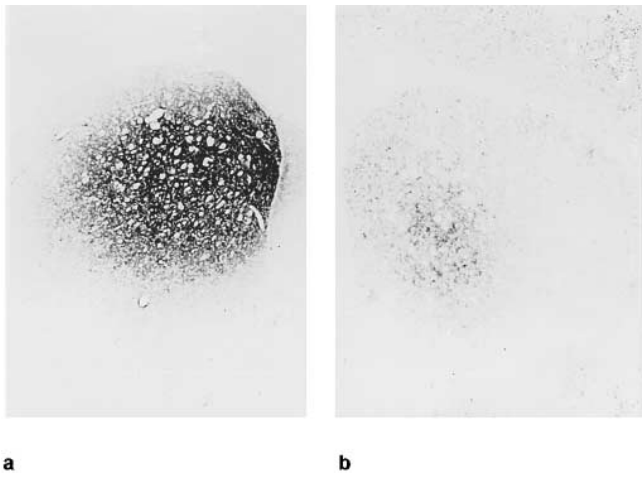

FIG. 4. Photomicrographs taken at a magnification of 25× from a coronal section at the level of the striatum showing tyrosinehydroxylase-immunoreactivity in unlesioned (a) and MPTP-treated animals (b).

amphetamine can still substantially increase the monoamines extracellular levels by a mechanism which mainly affects cytosolic dopamine. Though, amphetamine reportedly also enhances extracellular noradrenaline and serotonin levels, these effects are not accompanied with increased ambulation (1,16). Thus, it can be assumed that the amphetamine accessible pool of dopamine after MPTP toxicity is still fairly large. In support of this hypothesis we and others found only a modest increase of striatal ^3H spiroperidol binding (10). Therefore it would be of interest to measure extracellular dopamine levels in MPTP treated mice after an amphetamine challenge.

Further experiments with the D_1/D_2 agonist apomorphine suggest that a denervation-induced dopamine receptor supersensitivity might also be partially responsible for the hypermotility induced by dopaminomimetics. Behavioral effects of apomorphine in the dose we used are mediated by binding to postsynaptic dopamine receptors in the basal ganglia. The dopaminergic lesion after MPTP treatment seems to be accompanied mainly by a supersensitivity of the D_2 dopamine receptors. Furthermore, an upregulation of postsynaptic receptor binding sites is indicated by the increase of D_2 receptor binding sites using the D_2-specific ^3H-spiroperidol assay. This observation is consistent with that of Lau and Fung (10). Moreover it has been reported that enhanced locomotor activity after administration of dopaminergic agonists in mice after nigrostriatal lesions is mediated mainly by activation of D_2 dopamine receptors whereas the D_1 receptor seems to play a minor role (13). Similar results were found in MPTP-treated monkeys by Schneider et. al. (18) who described performance deficits and impairments of motor behavior which were improved by a treatment with D_2 agonists.

Previous studies indicated that different mice strains exhibit remarkably different susceptibilities to MPTP toxicity (6,19). Our data show that such strain differences are also found in the behavioral response to amphetamine, where C57Bl/6 black mice showed higher locomotor activity in response to the lower dose of amphetamine. However in general the behavioral outcome of MPTP-toxicity and subsequent treatments with dopaminomimetics was comparable between both strains. Thus, based on experience there is no need to

use a particular susceptible strain for psychopharmacological investigations in this subchronic mouse model.

In summary, MPTP-induced lesions in mice are followed by an upregulation of dopamine D_2 binding sites and an increased behavioral response to amphetamine. These data provide evidence for significant alterations of dopaminergic neurotransmission in the basal ganglia which are also accompanied by behavioral alterations. Therefore, the subchronic MPTP lesion in mice might serve as a useful model for investigations on the psychopharmacology of the partially denervated striatum.

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REFERENCES

1. Azzuro, A. J.; Rutledge, C. O. The release of endogeneous norepinephrine, dopamine and 5-hydroxytryptamine by amphetamine in various regions of the rat brain. Biochem. Pharmacol. 22:2801–2813; 1977.
2. Butcher, S. P.; Fairbrother, I. S.; Kelly, J. S.; Arbuthnott, G. W. Amphetamine -induced dopamine release in the rat striatum: an *in vivo* microdialysis study. J. Neurochem. 50:346–355; 1988.
3. Degryse, A. D.; Colpaert, F. E. Symptoms and behavioral features induced by 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) in an old Java monkey. Brain Res. Bull. 16: 561–571; 1986.
4. Gerlach, M.; Riederer, P.; Pruntek, H.; Youdim, M. B. H. MPTP mechanisms of neurotoxicity and their implications for Parkinson's disease. Eur. J. Pharmacol., Mol. Pharmacol. Sect. 208: 703; 1991.
5. Heikkila, R. E.; Sieber, B. A.; Manzino, L.; Sonsalla, P. K. Some features of the nigrostriatal dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in the mouse. Mol. Chem. Neuropath. 10:171–183; 1989.
6. Hoskins, J. A.; Davis, L. J. The acute effect on levels of catecholamines and metabolites in brain, of a single dose of MPTP in 8 strains of mice. Neuropharmacology 28:1389–1397; 1989.
7. Hu, S. C.; Chang, F. W.; Sung, Y. J.; Hsu, W. M.; Lee, E. H. Neurotoxic effects of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine in the substantia nigra and the locus coeruleus in BALB/c mice. J. Pharmacol. Exp. Ther. 259:1379–1387; 1991.
8. Kelly, P.; Iversen, S. D. Selective 6-OHDA-induced destruction of mesolimbic dopamine neurons: Abolition of psychostimulant-induced locomotor activity in rats. Eur. J. Pharm. 46:45–56; 1976.
9. Langston, J. W.; Ballard, P.; Tetrud, J. W.; Irwin, I. Chronic Parkinsonism in humans due to a product of mepiridine-analog synthesis. Science 219:979–980; 1983.
10. Lau, Y. S.; Fung, Y. K. Pharmacological effects of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) on striatal dopamine receptor system. Brain Res. 369:311–315; 1986.
11. Leysen, J. E. H.; Gommeren, W.; Laduron, P. M. Spiperone:a ligand of choice for neuroleptic receptors -1. Kinetics and characteristics of *in vitro* binding. Biochem. Pharmacol. 27: 307–316; 1978.
12. Lowry, O. H.; Roseburgh, R. N.; Farr, A. L.; Randall, R. J. Protein measurements with the folin phenol reagent. J. Biol. Chem. 193: 265–275; 1951.
13. Mierau, J.; Schingnitz, G. Biochemical and pharmacological studies on pramipexole, a potent and selective dopamine D_2 receptor agonist. Eur. J. Pharmacol. 215:161–170; 1992.
14. Mitra, N.; Mohanakumar, K. P.; Ganguly, D. K. Dissociation of serotonergic and dopaminergic components in acute effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mice. Brain Res. Bull. 28:355–364; 1992.
15. Ogawa, N.; Mizukawa, K.; Hirose, Y.; Kajita, S.; Ohara, S.; Watanabe, Y. MPTP-induced parkinsonian model in mice: biochemistry, pharmacology and behavior. Eur. Neurol. 26:16–23; 1987.
16. Peat, M. A., Warren, P. F.; Bakhit, C.; Gibb, J. W. The acute effects of methamphetamine, amphetamine and *p*-chloroamphetamine on the cortical serotonergic system in the rat brain: evidence for differences in the effects of metamphetamine and amphetamine. Eur. J. Pharmacol. 116:11–16; 1985.
17. Sayre, L. M. Biochemical mechanism of action of the dopaminergic neurotoxin 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP). Toxicol. Lett. 48:121–149; 1989.
18. Schneider, J. S.; Sun, Z. -Q.; Roeltgen, D. P. Effects of dopamine agonists on delayed response performance in chronic low-dose MPTP-treated monkeys. Pharmacol. Biochem. Behav. 48:235–240; 1994.
19. Sonsalla, P. K.; Heikkila, R. E. Neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and methamphetamine in several strains of mice. Prog. Neuro. Psychopharmacol. Biol. Psychiatry 12:345–354; 1989.
20. Sundström, E.; Strömberg, I.; Tsutsumi, T.; Olson, L.; Jonsson, G. Studies on the effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine on central catecholamine neurons in C56BL/6 mice. Comparison with three other strains of mice. Brain Res. 405: 26–38; 1987.
21. Zukin, S. R.; Young, A. B.; Snyder, S. H. Gamma-aminobutyric acid binding to receptor sites in the central nervous system. Proc. Natl. Acad. Sci. USA 71:4802–4807; 1974.