Title: TREATMENT FOR SCHIZOPHRENIA AND OTHER DOPAMINE SYSTEM DYSFUNCTIONS

Abstract

Methods and compositions for the treatment of positive and negative symptoms of schizophrenia and tardive dyskinesia are provided. Compositions include 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and its analogs, and/or pyridinium ions thereof, administered in amounts sufficient to reduce dopamine levels in subcortical areas of the brain without causing symptoms of Parkinson’s disease. One course of treatment can result in permanent or long-term amelioration of symptoms.
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TREATMENT FOR SCHIZOPHRENIA AND OTHER
DOPAMINE SYSTEM DYSFUNCTIONS

BACKGROUND OF THE INVENTION

Schizophrenia is a serious disease affecting one percent of the entire global population including about three million Americans. A permanent or long-term cure for this tragic disease would be of tremendous value to the human race. The symptoms of schizophrenia can be grouped into three separate categories. These are (1) positive symptoms related to hallucinations and reality distortion; (2) disorganized symptoms characterized by attentional impairment and thought disorder; and (3) negative symptoms such as apathy and loss of verbal fluency.

Dysfunction of the limbic-cortical system may be implicated in all three types of symptoms. Reduced excitatory glutamatergic inputs from the hippocampus and other limbic structures to the ventral striatum may be implicated in positive symptoms of psychosis and thought disorganization, and negative symptoms are likely to result from abnormal functioning of frontal lobe structures, e.g. those that receive connections from limbic structures, and/or anatomical irregularities. The dopamine hypothesis of schizophrenia associates the disease with increased activity in dopaminergic neurons. One of the strongest pieces of evidence for a dopamine disturbance in schizophrenia arises from the ability of D2 receptor antagonists to alleviate schizophrenic symptoms.

Effective antipsychotics acting on D2 receptors, including "typical" antipsychotics such as haloperidol and "atypical" antipsychotics such as clozapine, result in disruptions of the dopamine system. Persistence of negative symptoms often continues, even following neuroleptic treatment. The stability of negative symptoms has been, by some, attributed to the neuroleptic medications themselves. Long-term haloperidol treatment reduces the activity of dopamine cells in the substantia nigra. Clozapine reduces the activity of dopamine cells in mesolimbic/mesocortical cells in the ventral tegmental area that projects to the limbic system. More recent research has uncovered a multitude of abnormalities of the dopamine system itself and in its relation to other neurotransmitter systems in schizophrenia.
D1 receptor antagonism correlates highly with attenuated response in conditioned avoidance tasks that is a predetermined of neuroleptic efficacy. Also, researchers have demonstrated that D1 receptors located in the caudal portion of the striatum, when agonized, activate one of the strongest functional projections related to the auditory cortex. This may be a contributory source of auditory hallucinations. This sensory-neural pathway has not been fully researched. D3 receptor targeting medications are being evaluated for both their antipsychotic properties (antagonism) and Parkinsonian symptom alleviating (agonism) effects. Finally, D4 receptor antagonism has been demonstrated to restore prepulse inhibition (PPI), a sensory gating mechanism that is deficient in schizophrenia. Most medications fail to address all the symptoms that can be alleviated by reducing dopamine availability to all these mid-brain receptor subtypes. Dopamine antagonizing medications such as clozapine in psychosis-controlling doses occupy at least 70% of these receptors. To a certain degree, antagonism of all dopamine receptors (except D5 which is much more limited in expression) contributes to restorative effects in function.

There are four main dopaminergic pathways in the mammalian brain: (1) The mesocortical pathway runs from the ventral tegmental part of the mesencephalon to the frontal cortex, and is implicated in schizophrenia. (2) The mesolimbic pathway runs from the mesencephalon to the limbic areas such as the amygdala, hippocampus and nucleus accumbens (Nac) and is also implicated in schizophrenia. Along with the mesocortical pathway, the mesolimbic pathway arises in the ventral tegmental area (VTA) of the mesencephalon. (3) The nigral striatal pathway projects from its cell bodies in the substantia nigra (SN) to the striatum (ST) and is also implicated in schizophrenia (as well as being the one lesioned for the "Parkinson's model in lab animals). The nigral striatal pathway has excessive D2/D3 receptor sites. (4) The tuberoinfundibular tract runs from the hypothalamus to the anterior pituitary and has not been implicated in schizophrenia. It mediates the release of prolactin.
Reducing dopamine availability in the mesolimbic and striatal regions via depolarization block and receptor antagonism is not inherently compensated for by mesencephalic dopamine receptor upregulations. The up and down regulation of mRNA expression for dopamine receptors was mapped in response to haloperidol and clozapine. This study indicated no evidence of compensatory upregulation of mRNA activity in the basal ganglion. Several areas of the basal ganglia were demonstrated to have a reduction of mRNA activity in response to blockage of receptors and neural firing. Significant increases of dopamine receptor mRNA expression were observed in the cerebral cortex and temporal lobes. This may eventually contribute to the homeostasis of the cortical/subcortical circuitry. It seems there is an inverse reciprocal link between dopamine transmission in the frontal cortex and subcortical areas, especially the nucleus accumbens, which has both striatal and limbic components.

Conventional treatments for schizophrenia using neuroleptic dopamine receptor antagonists give rise to many side effects, some more severe than the illness itself, such as seizures, acute dystonia, drug-induced Parkinsonism, akathisia (inner restlessness and characteristic fidgety movements), tardive dyskinesia (involuntary movements such as chewing, lateral jaw movements, lip smacking and puckering), vermricular writhing and protrusions of the tongue, grimacing, forehead wrinkling, eye blinking and excessive winking and movements of the extremities, and irregular breathing and swallowing, and neuroleptic malignant syndrome (including seizures, dystonia and rigidity, fever, autonomic instability, delirium, myoglobinuria). Additional side effects include sexual dysfunction, urinary problems, hepatic dysfunction, ocular and dermatological problems, and cardiac and respiratory effects. Dopamine blocking by the neuroleptic medications results in an excess of prolactin, causing such side effects as decreased sexual interest, anorgasmia, amenorrhoea and the like.

Tardive Dyskinesia (TD) continues to be a significant clinical problem for both patients and doctors. New atypical neuroleptics were expected to eliminate the
development of TD, but currently the condition remains prevalent among patients with long term neuroleptic use. Cumulative five year prevalence rates are 20-26%, ten year prevalence rates are 49%, and 25 year rates are 68%.

In 1982, MPTP was introduced on the streets of California as a contaminant of a "synthetic heroin." A number of those who took the synthetic heroin in large amounts (4.5 grams in one reported case, 30 grams in a second, 16 ounces in a third, and one teaspoon per day for about a month in a fourth reported case) developed symptoms of severe Parkinson's Disease, but with no change in mental status. Neurotoxic effects appeared limited to damage to the substantia nigra. One individual who had taken low doses of MPTP showed significant destruction of nigrostriatal dopamine neurons; however, this patient had no symptoms of motor deficit or Parkinson's disease. A longitudinal follow-up of these individuals reported they are basically doing well and leading normal lives, albeit requiring daily Parkinsonian medications. There was no evidence of any cognitive deficits or peripheral damage (short- or long-term) due to large systemic exposure of MPTP other than a reported "burning sensation" during and shortly after the injection.

MPTP has been used as a model to simulate Parkinson's disease in animal models because it selectively destroys the small group of dopaminergic nerve cells in the substantia nigra of the brain which are also destroyed by degenerative processes in naturally-occurring Parkinson's disease.

MPTP is relatively harmless until converted into its active metabolite MPP+ by monoamine oxidase B (MOA B). MPTP, as well as being a substrate for MOA B, is also a mechanism-based inhibitor of this enzyme. On exposure to MOA B it is irreversibly converted into MPP+ and a small amount of MTDP+ is usually also formed (with a lesser degree of toxicity). After an intracranial injection of MPTP, one hundred percent recovery of this substance can be reobtained in its original and metabolite forms.
Symptoms of Parkinson's are not usually detected until about eighty percent of the dopamine-producing neurons have died. In primate models, it has been shown that dosages of MPTP in the range of .66 mg/kg or more are necessary before symptoms of motor dysfunction occur, that higher dosages over a longer period are necessary for severe nerve cell loss, and that considerable reduction of dopamine may occur without the development of clinical evidence of disordered motor function. For antipsychotic effects of neuroleptics to occur, it has generally been found necessary to induce about 70-89% D2 receptor blockade. However, neuroleptic dosages cause dopamine level reductions in much lesser amounts.

MPP+ is accumulated in the dopamine neuronal uptake system and concentrated within dopamine neurons, accounting for their selective destruction. Some destruction of cells involved in norepinephrine and serotonin also occurs. The mesolimbic dopaminergic pathway is about twice as resistant as the nigrostriatal dopaminergic pathway to MPTP toxicity.

When systemically injected, MPTP easily diffuses across the blood-brain barrier and, due largely to the high concentrations of MOA B in the walls of capillaries forming the blood-brain barrier, it is quickly converted to MPP+ and does not rediffuse back across into systemic circulation. On the other hand, a systemic injection of MPP+ is prevented from crossing the blood-brain barrier to exert neurotoxic effects due to the presence of the inherent quaternary pyridinium ion.

A method for treating schizophrenia which does not cause the serious side effects of neuroleptic drugs is needed. Such a treatment should be permanent, i.e. irreversible, or long-term.
SUMMARY OF THE INVENTION.

A method is provided to produce selective, controlled lesions of the dopamine systems in the midbrain in the regions most commonly antagonized by medications to alleviate positive symptoms of schizophrenia. Dopamine-producing cells are selectively destroyed to a level that ameliorates symptoms of schizophrenia, permanently or long-term, without causing Parkinson's-like symptoms, and without other undesirable side effects. This is accomplished by the administration of neurotoxic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or an effective analog, ion or salt thereof. These compounds attack dopamine-producing neurons, as well as some cells involved in norepinephrine and serotonin uptake. It is necessary to destroy about eighty percent of dopamine-producing neurons before symptoms of Parkinsons occur, as discussed above. So long as dosages are kept below amounts producing this extensive amount of damage, there is little danger of producing motor dysfunctions typical of Parkinson's disease when administering MPTP and related compounds. Reduction of dopamine levels throughout the brain is achieved at these dosages and provides observable amelioration of schizophrenic symptoms.

The treatment of this invention targets only subcortical dopamine neurons thus will not induce reductions of dopamine availability in hypofunctional areas of the frontal and temporal lobes that are also antagonized by neuroleptics. Thus, this treatment potentiates a restorative effect on cortical/subcortical circuitry and dopamine function.

The treatment permanently reduces positive symptoms of schizophrenia without chronic dopamine receptor antagonism, restoring homeostasis between the hypofunctional cortical areas in relation to the hyperfunctional subcortical areas of the schizophrenic brain. MPTP and related compounds act like high-powered neuroleptics but without the side effects, relapses, and non-compliance of known neuroleptic medications. Given in several controlled doses, they produce the same results which prescribed regimens of neuroleptics are aimed at achieving.
With respect to negative symptoms of schizophrenia which are persistent and stable even following conventional neuroleptic treatment that reduces positive symptoms, since neuroleptic medications that potentiate receptor upregulation in the hypofunctional cortical areas also antagonize those dopamine receptors the same way they antagonize the striatal and limbic dopamine receptors, restorative effects of cortical-subcortical circuitry that could alleviate this hypofunctionality is essentially impeded. Neuroleptic-treated individuals who have incurred this upregulation benefit by the procedure of this invention that specifically attenuates dopamine levels in the midbrain region, leading to alleviation of negative symptoms of schizophrenia.

The treatment of this invention using MPTP and related compounds for dopamine reduction reduces regional specific overactivity in the midbrain dopamine system. This also serves to restore some of the deficient frontal metabolic activity, allowing neurochemical messages sent from the higher cortical structures to the midbrain (and vice versa) to be adequately communicated. As the condition of schizophrenia has previously been treated, such a restoration of neurocircuitry was prevented from occurring. Neuroleptic medications inherently antagonize dopamine receptors both in cortical and subcortical areas, serving to “suppress” psychosis yet also impeding the transmission of dopamine chemical communication between cortical/subcortical areas. Midbrain-specific dopamine attenuation restores communication within these neural circuits, making this treatment much more efficacious than just another psychosis “suppressing” drug.

Since MPTP has both the ability to deplete dopamine like the dopamine depleters used to treat tardive dyskinesias and to restore metabolic activity in the globus pallidus and thalamic areas as discussed above, administration of MPTP provides alleviation of tardive dyskinesias.
Compounds useful in this invention are neurotoxic substrates for monoamine oxidase A and/or B known to the art, preferably those having the following general formula:

\[
\begin{array}{c}
A \\
\downarrow \\
B_n \\
N \\
R_1 \\
\end{array}
\]

where \(R_1\) is H, methyl, CH\(_2\)CCH, phenyl or benzyl;

A is substituted or unsubstituted phenyl, a substituted or unsubstituted, saturated or unsaturated, five- or six-membered heterocyclic carbon ring having S or O as a ring member, or a substituted or unsubstituted, saturated or unsaturated, five- or six-membered cycloalkyl ring;

\(n = 1\) or \(0\);

B is C or O;

ions thereof having a positively charged nitrogen (pyridinium ions thereof);

or pharmaceutically acceptable salts of the foregoing.

The active form of the compounds of Formula I are metabolites thereof which are pyridinium ions of the formula:
A class of preferred pyridinium ions comprises compounds of Formula II in which n is 0, and when A is phenyl, R₁ is phenyl, propyl or cyclopropyl; and in which when R₁ is methyl, A is cyclohexyl 3-cyclohexenyl, benzyl or N(CH₃)₂; and 4' methyl-MPP⁺.

Another class of preferred pyridinium ions comprises compounds of Formula II in which n is 0, A is phenyl and R₁ is CH₂CCH or benzyl. A further class comprises compounds of Formula II in which n is 0, A is tertbutyl and R₁ is methyl.

Preferred pyridinium ions include 2'-methyl and 4'-amino MPP⁺. Another preferred pyridinium ion is 4'-N(CH₃)₂-MPP⁺. A further preferred pyridinium ion is 1-methyl-4-phenylpyridine, the 4-phenyl isomer of MPP⁺. A further preferred pyridinium ion is 1-methyl-2-phenylpyridine, the 2-phenyl isomer of MPP⁺.

The MPTP analogs corresponding to the above preferred pyridinium ions form classes of preferred compounds of this invention.

These compounds may be directly administered to the patient. Suitable pharmaceutical salts of these ions may also be administered.
Another preferred class of compounds of this invention are compounds selected from the group consisting of those having the formulae:

\[
\begin{align*}
\text{III} & \quad \text{IV} \\
R_2 & \quad R_2 \\
R_3 & \quad R_3 \\
R_4 & \quad R_4 \\
R_5 & \quad R_5 \\
R_6 & \quad R_6 \\
\text{and} & \quad \text{and} \\
Y & \quad Y \\
\text{CH}_3 & \quad \text{CH}_3 \\
\end{align*}
\]

where \( R_2 \) is H or amino;

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\[ R_3 \text{ and } R_4 \text{ are independently } H, \text{CH}_3, \text{C}_2\text{H}_5, \text{X}, \text{or CX}_3; \]

where \( X \) is F, Cl, Br or I;

\[ R_5 \text{ and } R_6 \text{ are independently } H, \text{OH, OCH}_3 \text{ and } X; \text{ and} \]

\[ Y \text{ is S or O;} \]

pyridinium ions thereof; and

pharmaceutically acceptable salts of the foregoing.

A further preferred class of compounds are the MPTP analogs 2'-methyl-MPTP, 2'-fluoro-MPTP, 2'-chloro-MPTP, 3'-chloro-MPTP, 3'-bromo-MPTP and 1-methyl-4-t-
butyl-1,2,3,6-tetrahydropyridine; pyridinium ions thereof; and pharmaceutically acceptable salts of the foregoing.

Selective neural protective agents may be administered in combination with MPTP, its analogs, ions or salts, to prevent damage to dopaminergic neurons, or neurons other than dopaminergic neurons, e.g., those involved in norepinephrine and serotonin uptake.

In addition, toxicity enhancing agents may be administered in combination with MPTP, its analogs, ions or salts, to prevent axonal growth in dopamine-producing cells.

Dopamine upregulation agents or other antidotes may be administered as part of the treatment method of this invention if indicated after evaluation of the clinical effects of administration of the above compounds.

Pharmaceutical compositions suitable for intravenous administration to human patients comprising MPTP or its analogs, MPP⁺ or other pyridinium ions of such analogs, or pharmaceutically acceptable salts of the foregoing, in effective dosage amounts, as will be readily ascertainable to those skilled in the art. The term "intravenous administration" includes injection and other modes of intravenous administration.

Pharmaceutical compositions comprising combinations of MPTP, its analogs, pyridinium ions thereof, or pharmaceutical salts thereof, with neural protective agents, or toxicity-enhancing agents, or antidotes including agents that raise dopamine levels, preferably to pre-treatment amounts, and mixtures thereof are also included in this invention. Single dosages suitable for intravenous administration to a human of such compositions containing between about .14 and about 35 mg of active composition in a
pharmaceutical carrier suitable for intravenous administration to a human are also included in this invention.

The goal of the treatment method of this invention is to eliminate the need for patients to chronically take dopamine receptor blocking medications, thus completely avoiding the side effects which typically lead to noncompliance with treatment regimens. Coupling the administration of compositions of this invention to administration of dopamine level enhancers such as L-Dopa, which can be self-administered when the patient is having a bad day or depressed due to normal fluctuations in dopamine levels, will allow patients to return to normal functioning.

**DETAILED DESCRIPTION**

The structural formulae for MPTP and MPP+ are as follows:

![Structural formulae](image)

Analogs of MPTP and MPP+ as described above may also be used in this invention.

MPTP is commercially available, and any analogs which are not commercially available may be prepared by means known to the art.

Patients suitable for treatment by means of this invention are preferably patients who have not received previous treatments with dopamine receptor blockers.
Intervention as early as possible at onset of the illness could prevent progressive
dysfunction of neural circuitry which may occur later in the course of the disease.
Preferably, the treatment of this invention is administered prior to the third or fourth
schizophrenic episode. Patients who have been on long-term treatment with the "typical"
$D_2$ blocking neuroleptics such as haloperidol also benefit from treatment in accordance
with this invention. Depletion of dopamine, which can be accomplished through the
methods of this invention, results in alleviation of symptoms of tardive diskinesias
resulting from long-term use of "typical" blocking neuroleptics such as haloperidol.
Patients who have been treated with "atypical" blocking neuroleptics such as clozapine
also benefit from treatment. Patients who show good response to such "atypical"
neuroleptics but tend to be noncompliant with such treatment, or in denial, and/or subject
to chronic relapses, are good candidates for the treatments of this invention.

Therapeutically effective amounts of the pharmaceutical compositions of this
invention can be determined according to methods well-known to the art. The
compositions should be given in an amount sufficient to destroy dopamine-producing
cells in the brain such that dopamine production is diminished enough to cause a
clinically observable change in the symptoms being treated. However, the compositions
should not be administered in such large amounts as to cause symptoms of Parkinson's
disease such as motor impairment, rigidity or tremor. For example, suitable total dosage
amounts of between about .001 mg/kg and about .5 mg/kg are generally effective, or
typically between about .01 and about .2 mg/kg, depending on the severity of symptoms,
prior history of neuroleptic treatment, patient dopamine levels and other factors which are
taken into account by those skilled in the art. The dosages requires can be quite variable
from patient to patient. Patients may vary as to amounts of dopamine receptors, for
example as a result of long-term neuroleptic treatment, and assays to determine the level
of receptors may be used in calculating required dosages. The active neurotoxic
compositions in suitable pharmaceutical carriers are included in this invention. These
total dosage amounts are preferably split into two to five fractional doses for
administration in several doses, e.g. about two to five doses spaced about one to five days apart. Administration should be slow, preferably taking at least about fifteen minutes for each dose.

Since symptoms of Parkinson's are not observed until about eighty percent of dopamine-producing cells are destroyed, while much less cell destruction is required for observable decreases in dopamine production, there is little danger of accidentally overdosing patients. The treatment should achieve less than about eighty percent reduction in dopamine-producing neurons; and is preferably limited to destruction of less than about forty percent, or more preferably less than about twenty percent of dopamine-producing neurons.

To further minimize the risk of inducing motor disorders, it is preferred that the total dosage be given in timed increments, each dose being between about 2 ng/kg and about .25 mg/kg, more preferably between about .002 and about .1 mg/kg. After the first dose, an interval of one to several days should be allowed to observe symptoms, then a second dose given, followed by a similar observation period. The process may be repeated until symptoms have been maximally alleviated, so long as motor dysfunction does not occur. When symptoms have been satisfactorily ameliorated or completely eliminated, no further dosages need be given. If any symptoms typical of Parkinson's disease occur, further dosages should not be given, and antidotes should be administered as is more thoroughly discussed below.

Clinical evaluation of symptoms of schizophrenia can be done by those skilled in the art using art-known methods including standard tests such as the Brief Psychiatric Rating Scale (BPRS), tests of Latent Inhibition, experienced clinical observation, and patient verification of symptom reduction.
Reduction of dopamine in the subcortical regions does not typically result in substantial compensating production of dopamine receptor cells. Cortical areas have been reported as hypofunctional in schizophrenic patients. Thus, lesions in dopamine-producing areas will not inherently be compensated for by upregulation of dopamine receptor expression in subcortical regions, but may enhance dopamine effects in cortical areas where they are needed. It has been observed that blockading dopamine receptors in the subcortical areas has greatly upregulated dopamine receptor expression in regions which are hypofunctional in schizophrenic patients, such as the frontal cortex and parietal lobes. If patients do respond to damage to dopamine neurons by compensatory receptor upregulation in subcortical regions, this effect should be eliminated by second or subsequent treatments with the compositions of this invention.

The compositions of this invention may include or be administered in the form of pharmaceutically suitable salts as known to the art, e.g. hydrochloride, hydrobromides, phosphates, nitrates, perchlorates, citrates, lactates, tartrates, maleates, fumarates, tartrates, mesylates, esylates and sulfate salts. Such salts are formed by procedures well known in the art.

The compositions may be administered intravenously, orally, intraperitoneally, or by other means known to the art. Preferably the compositions are administered intravenously. For least risk to norepinephrine and serotonin uptake systems in the body, MPTP may be administered into the central nervous system (CNS) side of the blood-brain barrier, such as by spinal injection, where it is quickly converted to the active pyridinium ion which does not pass back through the blood-brain barrier. Preferably, the active compound is injected on the CNS side of the blood-brain barrier in the form of the pyridinium ion. Systemic injection to the body may also be done, as MPTP readily passes the blood-brain barrier where it is converted to the active metabolite MPP+ which does not pass back through the blood-brain barrier. When this systemic form of administration is done, neural protective agents may be administered in advance, e.g. one-
half hour or twenty minutes prior to injection of the active compound, to protect noradrenaline and serotonin uptake systems in the body.

The compositions may be mixed with suitable pharmaceutical carriers or excipients known to the art for the chosen dosage form. Such carriers suitable for intravenous administration of the compositions include sterile aqueous or non-aqueous solutions, suspensions or emulsions. Non-aqueous vehicles include propylene glycol, polyethylene glycol, vegetable oils such as olive oil and corn oil, gelatin and injectable organic esters such as ethyl oleate. Such dosage forms may also contain adjuvants such as preserving, wetting, emulsifying and dispersing agents.

The term "administering" includes all forms of introducing the active compositions of this invention into a patient's body, and includes prescribing or supplying treatment components to patients for self-administration.

Neural-protective agents for administration prior to or with the active neurotoxic compounds of this invention include desipramine, which protects against damage to cells responsive to noradrenaline, and citalopram which protects against damage to cells responsive to serotonin. Preferably the treatment of this invention is performed early in the course of the illness since long-term treatment with neuroleptics such as clozapine can severely block serotonin 5HT-2 receptors. When this has occurred it is important to use serotonin neural protective agents with this treatment.

Other types of neuroprotective agents which may selectively protect dopamine neurons in different parts of the brain, or which can be administered selectively to different parts of the brain, include 7-nitroindazole (7-NI) which selectively protects dopamine neurons in the nigral striatal dopaminergic pathway and is administered to compensate for the differential effect of the treatment on the ventral tegmental and nigral striatal areas and make dopamine production in these areas closer to equal. Gonadotropic
hormones, especially estrogen, similarly protect dopamine-producing neurons in the nigral striatal system.

Additional neuroprotective agents for dopamine neurons include selegiline (L-(-) -deprenyl and its metabolite L-(-)-desmethylselegiline, and 5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate (MK-801), deprenyl and other MAO-A and MAO-B inhibitors, mazindol, nomifensine, and pargyline, which can be administered selectively into areas other than the ventral tegmental areas, such as the cerebral cortex and nigral striatal areas, where protection is desired.

Guanethidine administered in advance of MPTP treatment has a protective effect on peripheral neurons. Administered in doses of about 10 mg/kg subcutaneously once a day three days before administration of MPTP, guanethidine protects peripheral neurons including epinephrine neurons without significantly interfering with reductions in dopamine levels.

The foregoing neural-protective agents for dopamine neurons can also be administered as antidotes to prevent progressive damage to dopamine neurons if adverse effects such as motor dysfunctions are observed after administration of the compositions of this invention.

Such neural protective agents should be administered in a dosage adequate to measurably lessen the toxic effects of MPTP on serotonin and norepinephrine-responsive cells, or on dopamine-producing cells, in amounts as is known to the art or may be readily ascertained by those skilled in the art. For example, dosages of manzidol may be administered in dosages of about 10 mg/kg, preferably about thirty minutes prior to administration of neurotoxic compositions of this invention.
Neurotoxicity enhancement agents include compounds which prevent regeneration of dopamine-producing neurons after they have been inactivated by MPP+ and related compounds. Acetaldehyde (ACE) and diethylthiocarbamate (DDC) assist in the retrograde transport of MPP+ from the axon of the neuron (where dopamine is produced) to the cell body. ACE is preferred as a relatively non-toxic agent, being the major metabolic product of ethanol metabolism. MPTP is more toxic to the nigrostriatal dopaminergic pathway than the mesolimbic which arises in the ventral tegmental area. Treatment with MPTP produces a twofold decrease in the number of dopamine neurons in the substantia nigra compared to that in the ventral tegmental area. However, the mesolimbic pathway is highly implicated in most types of schizophrenic symptoms as shown by the fact that the highly-effective antipsychotic clozapine reduces the number of active dopamine cells in mesolimbic/mesocortical cells in the ventral tegmental area that projects to the limbic system. Since MPTP converted to MPP+ is only about half as toxic to the ventral tegmental area than to the substantia nigra and dopamine lesions in the substantia nigra must be kept below the level of eighty percent cell destruction to avoid producing symptoms of Parkinsons, it may become important that any dopamine cell loss in the ventral tegmental area be preserved, and that these cells not be allowed to regenerate. If repeated MPTP treatments were required, the eighty percent limit on cell destruction to the substantia nigra could be exceeded in order to produce a clinically effective reduction in dopamine producing cells in the ventral tegmental area. Thus, administration of a toxicity-enhancing agent such as ACE or DDC may need to be undertaken concomitantly or shortly in advance of administration of MPTP or other neurotoxic compositions of this invention so as to achieve sufficient lesioning in the mesolimbic area to alleviate symptoms of schizophrenia without causing motor dysfunction.

Such toxicity enhancing agents should be administered in a dosage sufficient to prevent substantial regrowth of dopamine neurons, but not such a high dosage to be substantially toxic to other cells. For example, ACE is effective when administered at a
dosage of about 400 mg/kg approximately thirty minutes prior to administration of the neurotoxic compositions of this invention.

To further protect dopamine neurons in the nigral striatum, chloroquine and related antimalarial chloroquine compounds such as hydrochloroquine or chloroquine phosphate, and 4-aminoquinoline analogs, referred to herein as “chloroquine compounds,” having a high affinity to neuromelanin as known to the art may be used to bind to and protect dopamine neurons in the nigral striatum from MPTP damage. Intramuscular injection of 4 mg/kg chloroquine daily for a period of 24 days was able to prevent MPTP administered intravenously (0.35 mg/kg daily for four consecutive days) from exerting severe neurodegenerative or Parkinsonian effects in a primate model. Dosages in similar ratios to the MPTP administered as may be adjusted by those of skill in the art to substantially prevent striatal lesions while allowing therapeutic lesions in the mesolimbic system to occur should be used.

Striatal lesioning should not be totally prevented, however, as the striatum contributes to the overall production of dopamine. In addition, the auditory pathway in this area, as a source of auditory hallucinations, may be normalized by dopamine reduction in the striatum.

Pharmaceutical compositions comprising MPTP, an analog thereof, a biologically active ion of such compounds, and/or pharmaceutical salts of the foregoing, in combination with neural protective agents and/or toxicity enhancing agents are included within the scope of this invention.

If greater-than-desirable destruction of dopamine-producing cells results from administering the pharmaceutical compositions of this invention, a dopamine level enhancer, dopamine neuron protective agent or axon-regenerating agent may be administered as an antidote to the compositions of this invention to raise dopamine levels.
Dopamine level enhancers are known to the art and include L-Dopa, adenosine A (sub 2A) receptor antagonists, selegiline and zolpidem. Such dopamine level enhancers should be administered in a dosage sufficient to restore dopamine levels to those required to alleviate physical and adverse mental symptoms, but not such a high dosage as to counteract the effect of MPTP and lead to the original schizophrenic symptoms, such amounts being known to the art or readily ascertainable by those skilled in the art.

Axonal regeneration agents which may be administered if greater than desirable destruction of dopamine-producing cells results from administering the pharmaceutical compositions of this invention include deprenyl, which has been shown to be able to reverse MPP+-induced dopamine neural lesions by promoting new axonal growth and preventing cell death and brain-derived neurotrophic factor (BDNF), a member of the neurotrophic factor family, which has been found to increase the survival of dopamine neurons in embryonic mesencephalic culture.

Such axonal regeneration agents should be administered in a dosage sufficient to cause sufficient regeneration of dopamine-producing cells to provide a measurable increase in dopamine levels, but not such a high dosage as to completely counteract the effects of administration of MPTP.

Embryonic grafts may also be used as antidotes to counteract destruction of dopaminergic neurons.

Once elimination of positive, primary, especially psychotic symptoms is achieved and the patient becomes stabilized, a variety of approaches for continued rehabilitation may be more successfully employed. These include, but are not limited to: (1) the treatment of secondary symptoms such as depression, bipolar disorder, or anxiety; (2) psychological treatments, family therapy, vocational rehabilitation, etc.; (3) in treatment-resistant and/or negative symptomatic forms of this illness, selective serotonergic receptor
antagonizing agents, muscarinic receptor modulating chemicals, adrenergic receptor antagonists; and α7-nicotinic receptor agonists. These receptors appear to play a secondary role in some schizophrenic symptomology. Selective antagonism to the subclasses of serotonergic receptors (such as in the mechanism of several atypical neuroleptics), seems to facilitate the elimination of symptoms in the more difficult-to-treat forms of this illness. Other receptors have been shown to have modulating effects upon dopamine efflux. All classes of neuroleptics possess dopamine receptor blockading mechanisms to which diminished psychosis is attributed, but these mechanisms also seem to be the root of the more devastating side-effects in these patients. With the elimination of psychosis via the dopamine-reduction procedure described herein, isolation and pharmacological manipulation can be more successfully employed to treat the more subtle manifestations of this illness, without the inherent dopamine receptor antagonizing side-effects.

Side effects of the pharmaceutical compositions of this invention other than effects of accidental overdose do not present a problem because continued or repeat administration of the compositions is not expected to be necessary once the desired amelioration of symptoms has been achieved.

Example 1: Microdialysis Design

A total of 40 male C57 black mice are used to determine the effects of varying doses of MPTP + ACE on neurotransmitter and metabolite levels in the striatum. Each group has \( n = 10 \) subjects, with one group receiving ACE only (control) and the remaining three groups receiving injections of MPTP + ACE in 5, 10 and 15 mg/kg per day. The test subjects have a sample drawn each day prior to receiving another ACE or MPTP + ACE injection. Deprenyl, 0.25 mg/kg i.p., is administered following a maximum of five days of MPTP administration to evaluate dopamine re potentiation. Microdialysis probes are implanted to directly take these measures. Stereotaxic surgery is performed under 400 mg/kg i.p. of chloral hydrate anesthetic with metofane supplementation as necessary to
maintain surgical plane. Probes are implanted into the striatum using the following coordinates: 0.6 mm rostral, 2.4 mm lateral and 4.7 mm ventral to the bregma suture point (see Giovanni et al. [1994b], “Studies on species sensitivity to the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Part 2: Central administration of 1-methyl-4-phenylpyridinium,” *J. Pharmacology and Exp. Therapeutics* **270**:1008-1014). Probes are secured with cyanoacrylate and dental cement to assure their viability throughout the entire experiment.

Once implantation of microdialysis probes has been achieved, animals are exposed to a drug regimen intended to deplete dopamine in the striatum. On day 1 of the experiment, intraperitoneal (i.p.) MPTP in varying doses for each group is administered 10 minutes following the administration of 250 mg/kg ACE. An additional dosage of 250 mg/kg ACE is administered 20 minutes following the MPTP injection. Acetaldehyde has been previously shown to potentiate the effects of MPTP utilizing this 10 minute pre/20 minute post MPTP injection (Corsini et al. [1987], “1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity in mice is enhanced by ethanol or acetaldehyde,” *Life Sciences* **40**:827-832). This dosage pattern is utilized in all of the following experiments where MPTP is administered. Group 1 receives 250 mg/kg ACE i.p. followed 10 minutes later by an injection of saline, then 20 minutes later a second 250 mg/kg ACE dose i.p. is given. All experimental groups receive 250 mg/kg ACE 10 minutes prior to and 20 minutes following the administration of MPTP. Group 2 receives 5 mg/kg MPTP, group 3 receives 10 mg/kg MPTP, and group 4 receives 15 mg/kg MPTP. Observations are performed to observe motor effects of injections directly after injection protocol. Twenty-four hours later, samples are drawn from the microdialysis probes to measure levels of the monoamines and their metabolites (see below for specifics). Directly after samples have been drawn, animals receive injections identical to the previous day. This regimen is continued for a maximum of 5 days or until motor symptoms occur and do not resolve 24 hours after injections. If extrapyramidal symptoms do not resolve (see Corsini et al. [1987], *supra*), animals begin a daily regimen
of Deprenyl 0.25 mg/kg i.p. (see Lamensdorf et al. [1996], supra) to repotentiating dopamine levels. Again, samples are taken to track the repotentiation of the dopamine over the next several days. All mice are deeply anesthetized with chloral hydrate (400 mg/kg, i.p.) and decapitated. The brains are dissected to confirm the integrity of probe placement. Data from each specimen is considered valid that meets placement criteria or disregarded from further analysis in the event it does not.

Samples for microdialysis are drawn by a microdialysis pump. Dialysis samples are used to measure dopamine dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5HT) and 5-hydroxyindoleacetic acid (5HIAA) through High Pressure Liquid Chromatography. Values for each of these biochemicals are represented as ng/mg of protein and are corrected for recovery of external standards as previously described by Corsini et al. (1987), supra. From this data, doses and dose regimens that produce a 40%, 50%, 60% and 70% depletion of dopamine are noted and further utilized in the behavioral studies described below.

Daily dose dependent reductions followed by repotentiation levels of dopamine are evaluated on an individual subject basis. Overall dose response patterns for the treatment groups are combined and analysis of variance is used to identify between and within group effects. The data gathered from this experiment provide evidence of daily MPTP dosage/dopamine reduction patterns and demonstrate the repotentiation capacity of deprenyl. Feasibility is demonstrated by the achievement of consecutive MPTP dose-dependent dopamine reductions and significant repotentiation of dopamine levels. These data are then utilized in the behavioral and dye coupling experiments.

Example 2. MPTP Behavioral Response and Haloperidol Challenge.

Neuroleptics given in therapeutic doses are able to attenuate the conditioned avoidance response (CAR) in mice and rats. Prior research indicates that neuroleptic efficacy begins at a 50% binding rate of dopamine receptors in the brain. Following
conditioning, four MPTP treatment groups with 40%, 50%, 60% and 70% dopamine reduction levels (as predetermined by the microdialysis study) are evaluated for attenuated responses in the CAR test using pre/post lesion scores. An additional group given therapeutic amounts of haloperidol are used for comparison. The results show superiority in safety and/or efficacy of MPTP to haloperidol.

Task-Naïve mice are trained and tested in a one-way signaled avoidance test. The test uses a chamber with an elevated jump-stand and an electrified grid floor. During each trial a barrier that prevents access to the shelf between trials is retracted, and a buzzer sounds (4 seconds) to signal the arrival of an electric shock (10 seconds). When the floor is electrified the mice learn to jump to the shelf to avoid the shock during the test trials. Intertrial intervals are set at 20 seconds and the mice are given 20 consecutive training trials each session. Several sessions per day are given until each mouse achieves a correct response rate of at least 19 out of 20 trials. Mice are then randomly assigned to one of the five treatment groups.

Four groups of (n=5) mice are exposed to MPTP + ACE injections (as described above) which produce 40%, 50%, 60% and 70% depletion of dopamine levels in the striatum (as determined by the microdialysis study). An additional group (n=5) is administered haloperidol 1 ml/kg i.p., dissolved in lactic acid and pH adjusted to 6.0 with NaOH, in the comparison group. Twenty-four hours after the MPTP + ACE and haloperidol injection (comparison group), conditioned avoidance response is measured. Once the CAR response has been significantly attenuated in one of the MPTP treatment groups, the animals are administered deprenyl (0.25 mg/kg i.p.) to determine if the CAR response can be restored. Following this task, the MPTP treated animals demonstrating significant attenuation of the CARE response are completely withdrawn from deprenyl, sacrificed by cervical dislocation, and their brains removed and striatal areas analyzed for dopamine levels. All other animals are euthanized.
This experiment demonstrates that MPTP-induced dopamine can attenuate the conditioned avoidance response in a similar manner to what is observed with therapeutic doses of haloperidol.


Dye-coupling in mesencephalic regions, specifically in the nucleus accumbens (Nac) observed with the chronic administration of clozapine and haloperidol are evaluated in comparison with dye-coupling in MPTP-treated mice. Neuroleptics exert psychosis-eliminating effects on the dopamine system by the mechanisms of depolarization block (which inhibits dopamine neuron spike discharge) and dopamine receptor antagonism. This study looks at MPTP-induced dopamine reduction (which is similar to depolarization block) and evaluates if this causes a dye-coupling of neurons in the Nac and/or striatum similar to neuroleptics. Dye-coupling between neurons in the Nac is a plastic phenomenon that can undergo alteration with long-term neuroleptic administration. The increase in coupling in the motor-related caudate-putamen (CPu) seen with haloperidol but not clozapine may be related to the EPS-inducing profile of this drug. Both drugs have a similar effect on electronic coupling in the caudal accumbens shell, which may reflect their therapeutic action in the alleviation of positive schizophrenic symptoms. MPTP treatment is evaluated for the manifestation and/or absence of dye-coupling in comparison to observed patterns manifested by the above neuroleptics.

This experiment is a between-group comparison of dye-coupled neurons represented in a non-treated group, two neuroleptic treated groups, and an MPTP + ACE treated group. Similarities in neurophysiological responses of dye-coupling between neuroleptic groups and MPTP treated groups are evaluated. On day 1 of the drug administration phase, the MPTP + ACE group (n=10) is given a dose equivalent to that effective to attenuate the conditioned avoidance (determined above) or to evoke a 70% reduction of striatal dopamine availability (predetermined in the microdialysis study),
then free-fed and housed for the duration of 21 days. The haloperidol group (n=10) and clozapine group (n=10) respectively receive 0.50 mg/kg and 15 mg/kg i.p. per day for 21 days immediately preceding the experiment. A control group (n=10) receives a 21 day i.p. injection of saline. On day 22, all groups including n=10 untreated controls are sacrificed.

All mice are deeply anesthetized with chloral hydrate (400 mg/kg, i.p.) before decapitation. Brains are carefully removed and 400 μm thick sagittal slices containing the nucleus accumbens and dorsal striatum are cut while submerged in ice-cold physiological saline solution (124-mM NaCl, 5 mM KCl, 1.2 mM KH₂PO₄, 2.4 mM CaCl₂, 1.3 mM MgSO₄, 26 mM NaHCO₃, 10 mM glucose and saturated with 95%:5% O₂, CO₂) using a Vibratome. Micropipettes are pulled using a Flaming-Brown P-80/PC microelectrode puller and filled with the fluorescent dye Lucifer Yellow (10% in distilled water). After obtaining stable cell penetration, the dye is injected by applying constant hyperpolarizing current (-1.0 nA), interrupted by 10 ms-duration depolarizing pulses to prevent the tip from clogging. After completion of the experiment, the slices are fixed overnight in 10% buffered formalin, cleared in DMSO and later observed under an epifluorescence microscope (Leitz Orthoplan 2).

Slices containing both regions of the nucleus accumbens and the caudate-putamen (CPu) are obtained from all treatment groups (n=30) in which medium-sized (11-20 μm diameter) densely spinous neurons are successfully penetrated and labeled by intracellular injections of Lucifer Yellow. Since only medium spiny cells exhibit dye-coupling in the accumbens the analysis is limited to this cell type. Since previous studies have shown rostral vs. caudal differences in the modulation of dye-coupling in the accumbens shell, the results obtained from cells injected in the rostral third of the shell region are analyzed separately from those cells injected in the caudal third of the shell. Dye injections for periods of one minute or longer have been shown to consistently yield neurons with brightly stained stomata, dendrites and axons. Labeling of more than one cell is scored
as one case of dye-coupling. The number of injections yielding dye-coupling and the number of injections that labeled only single neurons are determined for the different treatment groups, along with the percent of injected cells showing coupling.

This experiment evaluates MPTP treated mice for the presence of dye-coupling, which is observed and considered an efficacious mechanism resulting from neuroleptic use. This experiment predicts that primates manifest dye-coupling in the accumbens as well. When dye-coupling is not evident at all in the MPTP treated group but the conditioned avoidance response is attenuated, this demonstrates a possibility of reduced EPS profile, similar to that of clozapine, over the classic neuroleptic haloperidol. Dye-coupling changes in all treatment groups is compared to control group values. Further analysis of differences between the neuroleptic groups and MPTP group are evaluated for significance.

Estrogen is known to preserve dopamine neurons from effects of MPTP, therefore, only male rodents are used.

Example 4. Time/Dose Controllability of Dopamine Reduction Levels.

Daily consecutive dose-dependent dopamine reduction parameters within the striatum are evaluated by continued administration of MPTP to non-human primates, in excess of therapeutic levels until extrapyramidal side effects (EPS) can be observed. Following this, an antidotal monoamine oxidase-A (MAO-A) inhibitor, l-Deprenyl, is evaluated for its efficacy in repotentiating dopamine to pretreatment levels and eliminating motor symptoms. Since dosages reported in the literature are based on non-human primate studies and there are individual differences in human and non-human primate susceptibility to MPTP, the initial dose is determined by evaluating the dopamine/dopamine receptor abnormalities on a per subject basis utilizing PET scans with radioligands. Following this, several smaller doses are administered to alleviate any residual symptoms.
For subjects already on neuroleptic medications, these are evaluated for compatibility with MPTP since some neuroleptic medications will not interfere with the effect of MPTP on midbrain dopamine neurons. Withdrawing patients from medications can be problematic. Thus the present treatment is administered in stages, the patient being given several incremental doses over time until a medication-free status can be achieved.

The repotentiation of dopamine to baseline values is not an intended normative part of this treatment. In fact, an intentional increase of dopamine levels to or above pretreatment levels would have a restorative or preserving effect on psychosis. The antidote to MPTP is given primarily to establish that there exists an effective antidotal therapy as an ultimate safeguard available to reverse the procedure.

Deprenyl is again administered to evaluate the restoration of pretreatment behavioral responses.

Example 5. Hyperprolactinemia evaluation.

Some neuroleptics with D2 receptor affinity have been known to cause hyperprolactinemia. Though MPTP has not been identified as selective for the tuberoinfundibular tract, pre-and post-MPTP treatment, prolactin levels are evaluated to ensure hyperprolactinemia will not be a factor in MPTP-treated patients.

This invention has been illustrated describing specific compositions and procedures. As will be evident to those skilled in the art, analogous compounds and methods may be substituted for those described herein within the scope of the appended claims.
CLAIMS

1. A method for ameliorating positive and negative symptoms of schizophrenia and
tardive dyskinesia comprising administering to a patient exhibiting such symptoms
an effective amount of a compound selected from neurotoxic substrates for
monoamine oxidase A or B having the following general formula:

```
  A
 /\  
B_n
 /\  
  N
 /\  
R_1
```

wherein $R_1$ is H or methyl, CH$_2$CCH, phenyl or benzyl;

$A$ is substituted or unsubstituted phenyl, a substituted or unsubstituted, saturated or unsaturated five or six-membered heterocyclic
ring having S or O as a ring member, or a substituted or unsubstituted,
saturated or unsaturated five- or six-membered cycloalkyl ring;

$n = 1$ or 0;

$B$ is C or O;

pyridinium ions thereof; and

pharmaceutically acceptable salts of the foregoing.

2. A method for ameliorating symptoms of schizophrenia comprising administering
to a patient exhibiting such symptoms an effective amount of a compound
selected from neurotoxic substrates for monoamine oxidase A or B having the following general formulae:

where $R_2$ is H or amino;
$R_3$ and $R_4$ are independently H, CH$_3$, C$_2$H$_5$, X, or CX$_3$;

where X is F, Cl, Br or I;
$R_5$ and $R_6$ are independently H, OH, OCH$_3$ and X; and
Y is S or O;
pyridinium ions thereof; and
pharmaceutically acceptable salts of the foregoing.

A method of claim 1 comprising administering an effective amount of a neurotoxic pyridinium ion selected from compounds having the following general formula:

wherein $R_1$, A, B and n are as defined above; and
pharmaceutically effective salts thereof.
4. The method of claim 3 comprising administering a compound in which \( n \) is 0 selected from the group consisting of compounds in which when \( A \) is phenyl, \( R_1 \) is phenyl, propyl, cyclopropyl, \( \text{CH}_2\text{CCH} \) or benzyl; compounds in which \( A \) is cyclohexyl, 3-cyclohexenyl, benzyl \( \text{N(CH}_3\text{)}_2 \) or tertbutyl, and \( R_1 \) is methyl; and 4'-methyl-MPP\(^+\); and corresponding MPTP analogs.

5. A method for ameliorating positive and negative symptoms of schizophrenia and tardive dyskinesia comprising administering to a patient exhibiting such symptoms an effective amount of a neurotoxic pyridinium ion selected from the group consisting of 2'-methyl MPP\(^+\); 4'-amino MPP\(^+\); 4'-\( \text{N(CH}_3\text{)}_2 \)-MPP\(^+\); the 1-methyl-2-phenylpyridine and 1-methyl-4-phenylpyridine.

6. A method of claim 1 comprising administering a compound selected from the group consisting of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP); 2'-methyl-MPTP, 2'-fluoro-MPTP, 2'-chloro-MPTP, 3'-chloro-MPTP, 3'-bromo-MPTP and 1-methyl-4-\( \text{t-butyl-1,2,3,6-tetrahydropyridine} \); pyridinium ions thereof; and pharmaceutically acceptable salts of the foregoing.

7. The method of claim 1 comprising administering MPTP, 1-methyl-4-phenylpyridinium ion (MPP\(^+\)) or a pharmaceutical salt of either.

8. The method of claim 1 wherein said compound, ion or salt is administered in combination with a selective neural protective agent selected from the group consisting of guanethidine, chloroquine compounds having high affinity to neuromelanin, desipramine, citalopram, 7-nitroindazole (7-NI), estrogen, selegiline (L(-) -deprenyl) L(-)-desmethyelselegiline, 5-methyl-10,11-dihydro-5\( \text{H-dibenzoz}[a,d]\)cyclohepten-5,10-imine maleate (MK-801), deprenyl, and other MAO-A and MAO-B inhibitors.
9. The method of claim 1 wherein said compound, ion or salt is administered in combination with a toxicity enhancing agent selected from the group consisting of acetaldehyde (ACE) or diethylthiocarbamate (DDC).

10. The method of claim 1 also comprising evaluating the clinical effects of administration of said compound, ion, or salt and administering a dopamine level enhancer or other antidote if indicated.

11. A pharmaceutical composition for ameliorating positive and negative symptoms of schizophrenia and tardive dyskinesia in patients exhibiting such symptoms comprising a neurotoxic substrate for monoamine oxidase A or B selected from the group consisting of:

\[
\begin{align*}
A \\
B_n \\
N \\
R_1
\end{align*}
\]

wherein \( R_1 \) is H or methyl, \( \text{CH}_2\text{CCH} \), phenyl or benzyl;

A is substituted or unsubstituted phenyl, a substituted or unsubstituted, saturated or unsaturated five or six-membered heterocyclic ring having S or O as a ring member, or a substituted or unsubstituted, saturated or unsaturated five- or six-membered cycloalkyl ring;

\( n = 1 \) or 0;

B is C or O;

pyridinium ions thereof; and

pharmaceutically acceptable salts of the foregoing.
in combination with a selective neural protective agent, or a toxicity-enhancing agent, or a combination thereof.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) : A61K 31/44
US CL. : 514/336, 318, 333, 256, 275
Accord to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
none

Electronic database consulted during the international search (name of database and, where practicable, search terms used)
databases: APS, HCAPLUS, WPIDS, MEDLINE
search terms: monoamine oxidase, schizophrenia, MPTP, MPP

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
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<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tr>
<td>Y</td>
<td>US 5,688,798 A (GODEL et al.) 18 November 1997, see the abstract.</td>
<td>1-7, 9, and 10</td>
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<td>Y</td>
<td>US 5,750,541 A (BYMASTER et al.) 12 May 1998, column 2, line 20 to column 3, line 11.</td>
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Further documents are listed in the continuation of Box C. [ ] See patent family annex.

Date of the actual completion of the international search: 26 SEPTEMBER 1999
Date of mailing of the international search report: 29 OCT 1999

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