Abstract:
The present invention relates to the efficient treatment of an individual afflicted with Parkinson's disease (PD), the instant treatment comprising administering to the individual an effective amount of (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof.
USE OF (R)-PHENYLPIRACETAM FOR THE TREATMENT OF PARKINSON'S DISEASE

FIELD OF THE INVENTION

[0001] The present invention relates to the efficient treatment of an individual afflicted with Parkinson's disease (PD), the instant treatment comprising administering to the individual an effective amount of (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof.

BACKGROUND OF THE INVENTION

[0002] This invention relates to a method of treating patients afflicted with Parkinson's disease.

[0003] Parkinson's disease (PD) is one of the most common chronic neurological diseases. The classical symptoms of PD are characterized by slowness of movement (bradykinesia/akinesia), rigidity and/or tremor. These symptoms may have an idiopathic, toxic, traumatic or genetic origin (e.g., in Parkinson's disease (PD)) or may also occur as a consequence of treatment, e.g., with dopamine receptor antagonists in schizophrenia (Parkinson syndrome). These movement disturbances can nowadays be treated rather effectively with various available PD drugs, particularly in early stages of the disease.

[0004] Currently, the principal symptomatic treatment for PD is based upon administration of dopamine-replacement therapy (e.g., levodopa (L-DOPA)) and/or therapy with dopamine receptor agonists (e.g., pramipexol). Long-term treatment with either therapy may lead to the development of dyskinesia, which is the most important motor complication that may arise in Parkinson patients. Although patients treated with dopamine receptor agonists are less prone to develop severe dyskinesia, other non-motor side effects such as somnolence, constipation, dizziness, nausea and hallucinations have been reported to be increased with this treatment option.
Concerning L-DOPA, long-term use of this treatment option leads to a reduction of its anti-parkinsonian efficacy and to the development of L-DOPA-induced dyskinesia (LID).

[0005] Additionally, in recent years it has been increasingly recognized that the current challenge of PD treatment are the so-called "non-motor" symptoms, for which satisfactory treatments are mainly lacking (Gallagher et al., Mov Disord. 25, 2493-2500, 2010). Amongst non-motor symptoms are autonomic dysfunctions (cardiovascular, urinary and gastrointestinal), sleep problems, psychosis, pain, cognitive deficits and fatigue. In clinical studies it could be demonstrated that fatigue and depression have the strongest association with a decline in the quality of PD patients' life (Beiske et al., Mov Disord. 25, 2456-60, 2010; Beiske and Svensson, Acta Neurol Scand Suppl.190, 78-81, 2010).

[0006] Fatigue in PD is multidimensional including physical, mental and general aspects (Havlikova et al., Parkinsonism Relat Disord. 14, 187-192, 2008; Havlikova et al., Eur J Neurol. 15, 475-80, 2008; Havlikova et al., J Neurol Sci. 270, 107-113, 2008). The physical dimensions of fatigue in PD are connected to problems regarding mobility and activity of daily living. Mental fatigue dimensions affect cognition, motivation, emotional well-being, and communication. In addition, general fatigue is related to bodily discomfort of the patients.

[0007] Fatigue associated with Parkinson's disease, as further defined below, has to be differentiated from fatigue symptoms in healthy individuals, where fatigue is a normal result of a natural reaction of body and mind to long-lasting and/or heavy burden, working, mental stress, over-stimulation or under-stimulation, jet lag, boredom, or lack of sleep. The physiological fatigue in healthy individuals is a normal response to physical exertion or stress, and its function is simply to protect the body from damage by overcharge. Such type of fatigue normally disappears spontaneously after a short recovery period. This type of fatigue isn't pathological and needs no therapeutic intervention. It is excluded from the intended medical use of the present invention.
Only the pathological disease-oriented type of fatigue is targeted by the present invention, and, unless otherwise defined, the term "fatigue" as used herein stands for the pathological form only.

A well-accepted measurement of this type of fatigue is the Fatigue Severity Scale (FSS), a self-administered unidimensional generic 9-item fatigue rating scale (Krupp et al., Arch Neurol. 46, 1121-1123, 1989). Each item has to be rated on a seven-grade Likert scale with a range from 1 (completely disagree) to 7 (completely agree), see Table 1. The total FSS score is mean score of the scores on respective 9 items. A total FSS score of 4 or higher present over 2 weeks prior to scoring is accepted as definition of presence of disease-oriented fatigue, especially in chronic diseases, e.g. Parkinson's disease.

Table 1: Fatigue Severity Scale

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>My motivation is lower when I am fatigued.</td>
</tr>
<tr>
<td>2</td>
<td>Exercise brings on my fatigue.</td>
</tr>
<tr>
<td>3</td>
<td>I am easily fatigued.</td>
</tr>
<tr>
<td>4</td>
<td>Fatigue interferes with my physical functioning.</td>
</tr>
<tr>
<td>5</td>
<td>Fatigue causes frequent problems for me.</td>
</tr>
<tr>
<td>6</td>
<td>My fatigue prevents sustained physical functioning.</td>
</tr>
<tr>
<td>7</td>
<td>Fatigue interferes with carrying out certain duties and responsibilities.</td>
</tr>
<tr>
<td>8</td>
<td>Fatigue is among the three most disabling symptoms.</td>
</tr>
<tr>
<td>9</td>
<td>Fatigue interferes with my work, family, or social life.</td>
</tr>
</tbody>
</table>

Treatment options for fatigue in PD are rather limited. Recently studies with approved PD drugs have been published that showed besides the main effects on motor symptoms also mediocre improvement of fatigue. Those drugs are the monoamine oxidase inhibitor rasagiline (Rascol et al., Lancet Neurol. 10, 415-23, 2011) and the dopamine agonist pramipexole (Morita et al., Intern Med. 50, 2163-2168, 2011). However, a specific drug with a strong anti-fatigue effect that is well tolerated by PD patients is so far not available.
Several studies about the effect of phenotropil in Parkinson's disease have been performed in Russia.

Karabanov et al. describe an open-label study of the effects of phenylpiracetam on patients suffering from PD (Karabanov et al., Atmosfera. Nervnye Bolezni 4, 29-32, 2009). In the study report, it is mentioned that fatigue was one of the parameters being monitored in the study, and a figure is presented showing a positive effect of phenylpiracetam on the combined parameter "general activity, physical and mental fatigue, asthenization symptoms". Karabanov neither presents data on the effect of phenylpiracetam on fatigue alone, nor differentiates between disease-related fatigue and "physical" fatigue. Karabanov et al. are stressing "asthenization", a term usually denoting a condition experienced by astronauts (healthy subjects) following long-term space flights, in which following return to Earth the astronaut experiences symptoms such as fatigue, irritability, lack of appetite and sleep disorders. Interestingly, this is deriving from the original use of the compound as "cosmic drug". (Mendonca et al., Mov. Disord. 22, 2070-2076, 2007).

Similarly, Kalinskij and Nazarov, describe the effect of phenylpiracetam on fatigability in the treatment of asthenic syndrome (Kalinskij and Nazarov, Zh Nevrol Psikhiatr Im SS Korsakova, 107, 61-63, 2007), and Akhapkina et al. provide additional data from a clinical study Efficacy of Phenotropil in the treatment of asthenic syndrome and chronic fatigue syndrome (Akhapkina et al., Atmosfera. Nervnye Bolezni 3, 28-31, 2004).

Vasiliev and Grigorjeva report the results of another study of phenylpiracetam in PD patients (Vasiliev, Y.N., and Grigorjeva, N.A., Siberian Medical Magazine, 2009). They report that the use of phenylpiracetam at dosages of 100 or 200 mg/day resulted in a decrease in neurological deficiencies, hypokinesia, and the level of depression, that its use prevented deterioration of sleep quality; and that phenylpiracetam in a dose of 100 mg/day quickly reduced the level of reactive anxiety; while it didn't affect mental abilities. The effect on the vegetative status was described as unambiguous.
Kolesnikova and Vasilyev analyzed the effect of phenylpiracetam on depression in PD, and concluded that phenylpiracetam contributed to a reduction of the level of depression (Kolesnikova, O.A., and Vasilyev, Y.N., Progress of Modern Natural Sciences 2008, 45-46).


Thus, despite the fact that many attempts have been made to (i) improve the current L-DOPA-based treatment scheme, and (ii) develop agents for the treatment of fatigue associated with Parkinson's disease, so far these attempts have had no clinically meaningful success in patients.

Thus, there is still a large unmet need to identify medicaments for improving the L-DOPA-based treatment of PD, and for the treatment of fatigue associated with Parkinson's disease.

The solution provided by the present invention to solve this problem, i.e. the use of a particular compound, has so far not been achieved or suggested by the prior art.

**SUMMARY OF THE INVENTION**

The present invention relates to a method of improving the dopamine-replacement therapy of Parkinson's disease and/or of treating fatigue, particularly mental fatigue, associated with Parkinson's disease in a subject in need thereof, comprising the step of administering an effective amount of (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof.

Thus, in a first aspect the present invention relates to a pharmaceutical composition comprising (R)-phenylpiracetam or a pharmaceutically acceptable salt
thereof for use in combination treatment with an agent for dopamine-replacement therapy of Parkinson's disease.

[0022] Another aspect of the invention relates to a pharmaceutical composition comprising (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof for use in the treatment of fatigue, particularly mental fatigue, associated with Parkinson's disease.

[0023] In another aspect the present invention relates to a method of treating fatigue associated with Parkinson's disease in a subject in need thereof, comprising the step of administering an effective amount of (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof.

[0024] In another aspect the present invention relates to a method of treating fatigue associated with Parkinson's disease in a subject in need thereof, comprising the step of administering an effective amount of (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] Figure 1 shows that (R)-phenylpiracetam increases extracellular dopamine levels in rat striatum as shown by brain microdialysis.

[0026] Figure 2 shows the concentration of (R)-phenylpiracetam in the brain, as determined by brain microdialysis, after intraperitoneal (i.p.) application. Affinity for DA transporter is c.a. 13 μM.

[0027] Figure 3 shows that (R)-phenylpiracetam increases locomotor activity (horizontal activity) in rats (administration i.p. 15 min before test) (*: p< 0.05 vs. vehicle, Kruskal-Wallis one-way ANOVA on ranks at each time interval followed by rank sum test).
[0028] **Figure 4** shows the CNS profile of (R)-phenylpiracetam in an electroencephalography (EEG) screen.

[0029] **Figure 5** shows the results from a progressive ratio test for determining the influence of (R)-phenylpiracetam on motivation in comparison to amphetamine.

[0030] **Figure 6** shows the results from a progressive ratio test for determining the influence of (R)-phenylpiracetam on motivation in comparison to methylphenidate.

[0031] **Figure 7** shows the results from a cost benefit test for determining the influence of (R)-phenylpiracetam on motivation in comparison to amphetamine.

[0032] **Figure 8** shows the results from a cost benefit test for determining the influence of (R)-phenylpiracetam on motivation in comparison to methylphenidate.

[0033] **Figure 9** shows the effect of (R)-phenylpiracetam on rotation in rats with unilateral SNc lesion (model of Parkinson's Disease).

[0034] **Figure 10** shows the effect of (R)-phenylpiracetam on rotation produced by L-DOPA in rats with unilateral SNc lesion (model of Parkinson's Disease). (R)-phenylpiracetam enhances L-DOPA rotations (90-150 min) but when L-DOPA effects disappear, it produces ipsilateral rotations (270-360 min). Both contra- and ipsilateral rotations indicate antiparkinsonian activity based on postsynaptic and presynaptic mechanisms, respectively.

[0035] **Figure 11** shows the effect of (R)-phenylpiracetam on dyskinesia produced by L-DOPA in rats with unilateral SNc lesion (model of Parkinson's Disease). (R)-phenylpiracetam did not enhance L-DOPA dyskinesia.
Figure 12 shows the effect of (R)-phenylpiracetam on hypokinesia produced by reserpine (5 mg/kg) + alpha-methyl-p-tyrosine (250 mg/kg) (model of Parkinson's Disease).

Figure 13 shows the effect of (R)-phenylpiracetam on hypokinesia produced by haloperidol (0.2 mg/kg) (model of Parkinson's Disease).

Figure 14 shows that (R)-phenylpiracetam does not influence L-DOPA concentration in the brain after i.p. application as assessed using brain microdialysis.

Figure 15 shows that L-DOPA does not influence (R)-phenylpiracetam concentration in the brain after i.p. application as assessed using brain microdialysis.

Figure 16 shows the effect of (S)-phenylpiracetam on hypokinesia produced by haloperidol (0.2 mg/kg) (model of Parkinson's Disease) at 50 mg/kg (Fig. 14A) and at 100 mg/kg (Fig. 14B).

DETAILED DESCRIPTION OF THE INVENTION

The peculiarity of this invention compared to former treatment approaches for treating Parkinson's disease and/or fatigue associated with Parkinson's disease is the so far unknown therapeutic efficacy of (R)-phenylpiracetam, which is presumably based at least in part on the newly identified activity of (R)-phenylpiracetam as the dopamine re-uptake inhibitor.

Thus, the present invention relates to the use of (R)-phenylpiracetam and any of its salts, solvates and conjugates, which possesses at least an inhibitory activity on the dopamine re-uptake transporter.
[0043] Thus, in a first aspect the present invention relates to a pharmaceutical composition comprising (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof for use in combination treatment with an agent for dopamine-replacement therapy of Parkinson's disease.

[0044] The term "phenylpiracetam" is known in the art and refers to the compound 2-(4-phenyl-2-oxopyrrolidin-1-yl)acetamide (C12H14N2O2; MW 218.3 g/mol). Phenylpiracetam is also known as fenotropil, phenotropyl, phenotropil, fenotropyl, or carphedon and was developed in Russia, where it is available as a prescription medicine under the name "Phenotropil®". As used herein, phenylpiracetam refers to the substance, as well as its pharmaceutically acceptable salts.

[0045] Phenylpiracetam is optically active and is available as a racemate of the two enantiomers. The International Nonproprietary Name (INN) "Fonturacetam" has been assigned to racemic phenylpiracetam. An international patent application with priority date in 2006 first disclosed the separation of the two enantiomers and demonstrated that the (R)-enantiomer is predominantly responsible for the pharmacological activity (WO 2007/104780). (R)-phenylpiracetam showed more pronounced activity in animal models for detecting antidepressant, analgesic, muscle relaxant and psychostimulant effects. The patent claims (R)-phenylpiracetam for the use as an antidepressant, as a stress-protective agent, as a modulator of locomotor activity, as a muscle relaxant and as an analgesic.

[0046] More detailed pharmacological data of (R)-phenylpiracetam have been published recently by the same authors (Zvejniec et al., Basic Clin Pharmacol Toxicol. 109, 407-412, 2011). In the open-field test a significant increase in locomotor activity was found, which was just slightly stronger with the (R)-phenylpiracetam than with (S)-phenylpiracetam. Also in the forced swim test, used as a model for depression, (R)-phenylpiracetam was also only slightly more potent than (S)-phenylpiracetam. However, in the passive avoidance test (R)-phenylpiracetam enhanced memory function significantly better compared to (S)-phenylpiracetam. The authors conclude that these results may be important for the clinical use of optically pure isomers of phenylpiracetam.
Prior to the surprising finding of the present inventors that (R)-phenylpiracetam acts as a dopamine re-uptake transporter inhibitor, the precise pharmacological mechanism of action of (R)-phenylpiracetam had not been elucidated. In pharmacological studies phenylpiracetam was found to activate the operant behaviour, to counteract psychodepressant effects of diazepam, to inhibit post-rotational nystagmus, and to prevent the development of retrograde amnesia. It also exhibited anticonvulsant action (Bobkov et al., Biull Eksp Biol Med. 95, 50-53, 1983) and some neuroprotective activity in experimental cerebral ischemia (Tiurenkov et al., Eksp Klin Farmakol. 70, 24-29, 2007). Thus, phenylpiracetam exhibits additional pharmacological effects, which are not yet fully identified and are differentiating phenylpiracetam from other pure dopamine re-uptake transporter inhibitors.

In humans phenylpiracetam is administered orally and shows a half-life of 3-5 hours. There are only a small number of low-scale exploratory clinical trials predominantly published in Russian journals. They have shown possible links between intake of phenylpiracetam and improvement in a number of conditions and diseases including asthenic syndrome and autonomic disturbances in brain trauma (Kalinskij and Nazarov, Zh Nevrol Psikhiatr Im S S Korsakova.107, 61-63, 2007), brain organic lesions (Savchenko et al. Zh Nevrol Psikhiatr Im S S Korsakova.105, 22-26, 2005), epilepsy (Bel'skaia et al. Zh Nevrol Psikhiatr Im S S Korsakova.107, 40-43, 2007, Lybzikova et al., Zh Nevrol Psikhiatr Im S S Korsakova.108, 69-70, 2008), stomatological problems (Novikova et al. Stomatologiiia (Mosk).87, 41-45, 2008), and vascular encephalopathy (Gustov et al. Zh Nevrol Psikhiatr Im S S Korsakova.106, 52-53, 2006). Effects of phenylpiracetam on immunological consequences of stroke have also been described (Gerasimova et al., Zh Nevrol Psikhiatr Im S S Korsakova.105, 63-64, 2005).

According to the package insert of the drug as approved in Russia, phenylpiracetam is a nootropic drug, which has an expressed anti-amnesic action, a direct activating effect on the integrative activity of the brain, helps consolidate memory, improves concentration and mental performance, facilitates the learning process, increases the information transfer between the hemispheres of the brain, increases the resistance of brain tissue to hypoxia and toxic effects, has anticonvulsant and anxiolytic
effects, regulates the processes of activation and inhibition of central nervous system, and improves mood. It is furthermore stated that phenylpiracetam has a positive effect on the metabolism and blood circulation in brain, stimulates the redox processes and increases energy potential through utilization of glucose, improves regional blood flow in ischemic areas of the brain. It increases noradrenaline, dopamine and serotonin content in the brain, does not affect the levels of GABA, associates neither with GABAA nor GABAB receptors, has no noticeable effect on the spontaneous bioelectric activity of the brain, does not influence respiration and the cardiovascular system. It shows no significant diuretic effect and has anorexigenic effect during treatment. According to the Russian package insert, the stimulating effect of phenylpiracetam manifests in the ability to provide a moderate effect on motor responses, to enhance physical performance. The moderate psychostimulant effect of the drug is combined with an anxiolytic activity, and it improves mood, has some analgesic effect and raises the threshold of pain. The adaptogenic effect of phenylpiracetam is manifested in increasing resistance to stress in conditions of excessive mental and physical overload, fatigue, hypokinesia and immobilization, and at low temperatures.

[0050] As shown above in sections [0012] to [0016], phenylpiracetam has been tested in some clinical studies in patients suffering from Parkinson’s disease. While the publications listed above report some positive effects on certain symptoms associated with Parkinson’s disease, no clear picture about potentially beneficial applications of phenylpiracetam emerged from these studies.

[0051] While (R)-phenylpiracetam was synthesized and described in the literature as nootropic compound, the present applicants have surprisingly found that (R)-phenylpiracetam has features not fitting into class of nootropics.

[0052] The term nootropics was created in 1960s, with the discovery of piracetam followed by pramiracetam, oxiracetam, aniracetam, tenilsetam and others, and is nowadays often used to describe cognitive enhancers that are neuroprotective and non-toxic. Most nootropics do not have a clearly defined mechanism of action and work, and seem to exert their cognitive and/or neuroprotective effects through changes in brain blood flow, energy management etc.
In contrast to nootropics, (R)-phenylpiracetam appears to have a clearly defined mechanism of action by inhibition of dopamine (DA) uptake, and behavioural effects are seen at the doses that create in the brain levels within affinity for DA uptake seen *in vitro* and produce an increase in the DA concentration in the brain as assessed by brain microdialysis (20 µM at 50 mg/kg with an affinity constant of 6 µM; see Examples).

Furthermore, in the EEG pattern there is a clear difference between nootropics and (R)-phenylpiracetam. For nootropics, a decrease of power for nearly all waves can be shown in the reticular formation, while there is no change of theta, delta, alphas and beta2 waves for (R)-phenylpiracetam (see Examples).

Additionally, (R)-phenylpiracetam acts more potently in animals, which have been treated to induce symptoms resembling Parkinson's disease (called here diseased or parkinsonian animals) than in normal animals. Therefore (R)-phenylpiracetam appears to be more suitable to correct deficits e.g. fatigue specific for PD than general everyday fatigue.

Finally, it could be shown that (R)-phenylpiracetam apparently enhances L-DOPA effects in the medium to long term range which is not due to pharmacokinetic interactions since brain levels of either L-DOPA or (R)-phenylpiracetam are not higher in animals treated with both substances as compared to single treatment.

The phrase "pharmaceutically acceptable", as used in connection with compositions of the invention, refers to molecular entities and other ingredients of such compositions that are physiologically tolerable and do not typically produce untoward reactions when administered to a mammal (e.g., human). The term "pharmaceutically acceptable" may also mean approved by a regulatory agency of the federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in mammals, and more particularly in humans.

The term "salt" is defined as a chemical containing different charged components. The term salt also includes hydrates and solvates.
(R)-Phenylpiracetam may be used according to the invention in the form of any of pharmaceutically acceptable salts, solvates and conjugates. Any references to (R)-phenylpiracetam in this description should be understood as also referring to such salts, solvates and conjugates.

In the context of the present invention, the term "dopamine-replacement therapy" refers to the principal symptomatic treatment for PD that is based upon administration of either (i) an agent replacing, or increasing the level of, endogenous dopamine (e.g., levodopa (L-DOPA)), or (ii) of a dopamine receptor agonist (e.g., apomorphine). Long-term treatment with either therapy may lead to the development of dyskinesia, which is the most important motor complication that may arise in Parkinson patients. Although patients treated with dopamine receptor agonists are less prone to develop severe dyskinesia, other non-motor side effects such as somnolence, constipation, dizziness, nausea and hallucination have been reported to be increased with this treatment option. Concerning L-DOPA, long-term use of this treatment option leads to a reduction of its anti-parkinsonian efficacy and to the development of L-DOPA-induced dyskinesia (LID).

LID is characterised by a mixture of choreiform, dystonic or ballistic/myoclonic movements that are observed after L-DOPA administration. It is reported that about 30% of the Parkinson patients will experience dyskinesia after 4-6 years of treatment with L-DOPA while close to 90% will suffer from this complication after 9 years. Although the cause of dyskinesia remains unknown, the main risk factor for the development of LID is young age at PD onset, the disease severity and duration as well as a high initial dose of L-DOPA treatment. Ultimately, this complication severely impairs the quality of life and well-being of the patient and therefore limits the use of this drug as most important therapeutic agent.

According to the literature, LID may be related to the pulsatile and intermittent nature of L-DOPA therapy. Upon systemic administration of L-DOPA to Parkinson patients, dopamine is formed, stored and released by the remaining dopaminergic terminals as well as by other cellular components present in the striatum such as serotonergic neurons. Since the striatal serotonin system remains relatively spared in
most PD patients, it is believed to play an important role in determining the efficacy of L-
DOPA therapy.

[0063] The term "treat" is used herein to mean to relieve or alleviate at least one symptom of a disease in a subject. Within the meaning of the present invention, the term "treat" also denotes to arrest, delay the onset (i.e., the period prior to clinical manifestation of a disease) and/or reduce the risk of developing or worsening a disease. Thus, "treatment" as used herein includes modifying, curative and symptomatic treatments.

[0064] As used herein, the term "subject" encompasses mammals including animals and humans.

[0065] In particular embodiments, said agent is L-DOPA.

[0066] In particular embodiments, the amount of said agent used in said combination treatment is reduced compared to the amount administered in the treatment cycle prior to said combination treatment.

[0067] In particular embodiments, said agent is administered in said combination treatment at a reduced frequency compared to the frequency of administration in the treatment cycle prior to said combination treatment.

[0068] In particular embodiments, said agent is L-DOPA. In particular such embodiments, L-DOPA is administered once daily.

[0069] In particular embodiments, (R)-phenylpiracetam is for use in a treatment comprising the following steps:
(a) administering (R)-phenylpiracetam to a patient under dopamine-replacement therapy in combination with said agent administered for dopamine-replacement;

(b) reduction of said agent in daily increments of 10% until signs of Parkinson's symptoms re-emerge; and

(c) increase of said agent once by 10% compared to the amount of said agent last administered according to step (b); and

(d) continuation of the combination treatment using (R)-phenylpiracetam and the amount of said agent used in accordance with step (c).

[0070] In particular embodiments, said steps are performed in order to establish said combination treatment.

[0071] In particular embodiments, said steps are performed in order to improve an established combination treatment.

[0072] Another aspect of the invention relates to a pharmaceutical composition comprising (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof for use in the treatment of fatigue, particularly mental fatigue, associated with Parkinson's disease.

[0073] In the context of the present invention, the term "fatigue associated with Parkinson's disease" refers to pathological fatigue that is independent of a type of fatigue, which is a normal response to physical exertion or stress (known as peripheral fatigue). This peripheral fatigue refers to muscle fatigue and is induced by repetitive muscle contractions (e.g. after athletics sports) (Chaudhuri and Behan, J Neurol Sci. 179(Suppl. 1-2), 34-42, 2000; Chaudhuri and Behan, Lancet. 363, 978-88, 2004). Disease-orientated fatigue is not caused by muscle overuse or physical impairment outside the central nervous system. This central fatigue is a subjective feeling with
symptoms of disease-associated mental fatigue (a subjective feeling of having impaired concentration, reduced memory, and speech difficulties) and disease-associated physical fatigue (subjective feeling of being exhausted and lacking energy). This subjective disease-associated fatigue can have its origin in particular embodiments by disturbance of the dopaminergic system of the CNS, particularly a lack of dopamine, and potentially also homeostatic changes, which lead to an abnormal degree of persistent tiredness, weakness or exhaustion.

[0074] While phenylpiracetam has apparently been prescribed *inter alia* for the treatment of stress associated with fatigue in healthy patients, as described in sections [0045] and [0049] above, phenylpiracetam has hitherto not been associated with the treatment of fatigue, particularly mental fatigue, associated with Parkinson's disease.

[0075] As reported above in Section [0012], Karabanov et al. reported certain observations related to the effect of phenylpiracetam on fatigue, but did neither differentiate between disease-related fatigue and "physiological" fatigue, and instead focussed on "asthenization". In contrast, the so-called "Parkinson Fatigue" is an outstanding embodiment of disease-associated fatigue seen exclusively in PD patients. Using common rating scales for fatigue causes problems in PD patients, as the critical assessment of Friedman JH et al (Mov. Disorders 25, 805-822, 2010) revealed. The "Parkinson Fatigue Scale" developed by Brown RG et al. (Parkinsonism Related DisordersH, 49-55, 2005) excludes emotional and cognitive features that may occur as part of the fatigue experience but which may also occur independently in Parkinsonism. The 16 specific items of this scale are reported by the affected patients themselves and allow a clear separation of the Parkinson-specific type of fatigue from other types of fatigue, wherein the cut-off point is at 7 (binary calculation).

[0076] A well-accepted measurement of this type of fatigue is the Fatigue Severity Scale (FSS), which has been described above.

[0077] In particular embodiments, the pharmaceutical composition is for use in the treatment of fatigue with a score on the FSS of at least 4, particular with a score of at least 4 for at least 2 weeks.
In particular embodiments, the pharmaceutical composition is for use in the treatment of fatigue with a score on the Parkinson Fatigue Scale of at least 7.

In particular embodiments of the methods of the present invention, (R)-phenylpiracetam is present as (R)-phenylpiracetam hydrochloride.

In particular embodiments of the methods of the present invention, (R)-phenylpiracetam is for administration in a range from about 1 mg to about 250 mg/day, or in a range from about 25 mg to about 200 mg/day, or in a range from about 50 mg to about 150 mg/day.

In the context of the present invention, the term "about" or "approximately" means between 90% and 110% of a given value or range, i.e. "about 100" means "between 90 and 110". In narrower embodiments, the term "about" or "approximately" means between 95% and 105% of a given value or range, or between 98% and 102% of a given value or range, or between 99% and 101% of a given value or range.

In particular embodiments of the methods of the present invention, (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof is for administration once a day (s.i.d.), twice a day (b.i.d.), or three times a day (t.i.d.).

In particular embodiments of the methods of the present invention, (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof is for administration in an oral formulation.

In particular embodiments of the methods of the present invention, (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof is for administration in combination with at least one additional pharmaceutical agent which has been shown to be effective for the treatment of Parkinson's disease.
In particular embodiments, said at least one additional pharmaceutical agent is selected from: L-DOPA, carbidopa, other types of dopamine agonists, eltoprazine, MAO-inhibitors, COMT inhibitors, and benserazide. In particular embodiments, said at least one additional pharmaceutical is L-DOPA, a combination of L-DOPA and carbidopa, or a combination of L-DOPA and benserazide.

In particular embodiments of the methods of the present invention, (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof is for administration in combination with at least one additional pharmaceutical agent which has been shown to be effective for the treatment of fatigue, particularly mental fatigue, associated with Parkinson's disease.

In particular embodiments, said at least one additional pharmaceutical agent is selected from: rasagiline and pramipexole.

In another aspect the present invention relates to a method of treating Parkinson's disease in a subject in need thereof, comprising the step of administering a therapeutically effective amount of (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof.

The term "therapeutically effective" applied to dose or amount refers to that quantity of a compound or pharmaceutical composition sufficient to result in a desired activity upon administration to a mammal in need thereof.

In particular embodiments, (R)-phenylpiracetam is enhancing the efficacy of a dopamine-replacement therapy.

In a particular embodiment, said dopamine-replacement therapy is the treatment with L-DOPA.
[0092] In certain embodiments, the treatment relates to the treatment of one or more motor symptoms of PD, particularly wearing off, on-off phenomenon, and "end-of dose" dystonia.

[0093] In certain embodiments, the treatment relates to reducing and/or delaying the onset of levodopa-induced dyskinesia (LID).

[0094] In another aspect the present invention relates to a method of treating fatigue associated with Parkinson's disease in a subject in need thereof, comprising the step of administering an effective amount of (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof.

[0095] In particular embodiments, said fatigue is mental fatigue associated with Parkinson's disease.

[0096] In particular embodiments, the invention relates to a method of treating fatigue with a score on the FSS of at least 4, particular with a score of at least 4 for at least 2 weeks.

[0097] In particular embodiments, the invention relates to a method of treating fatigue with a score on the Parkinson Fatigue Scale of at least 7.

[0098] In certain embodiments, the treatment of fatigue relates to the treatment of fatigue-associated symptoms in PD, particularly inactivity, motivational-deficit, floppiness, exhaustion, lassitude, and prostration.

[0099] In certain embodiments, the present invention relates to a method of treating sleep-associated problems of PD patients, which have significant impact on their quality of life, particularly general tiredness, and drowsiness.

[00100] (R)-phenylpiracetam may be administered as a single anti-fatigue agent or in combination with one or more additional pharmaceutical agents for the therapy of fatigue, particularly mental fatigue, associated with Parkinson's disease.
In particular such embodiments, said one or more additional pharmaceutical agents are selected from rasagiline and pramipexole.

In certain embodiments of the method of the present invention, (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof is administered in a range from about 1 mg to about 400 mg/day.

In a further embodiment of the method of the present invention, (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof is administered in a range from about 25 mg to about 350 mg/day.

In a still further embodiment, (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof is administered in a range from about 50 mg to about 300 mg/day, particularly in a range from about 50 mg to about 150 mg/day.

In yet another embodiment of the method of the present invention, (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof is administered once a day, particularly about 200 mg once a day.

In yet another embodiment of the method of the present invention, (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof is administered in a multiple dose, for example twice a day, or three times a day, particularly twice a day, particularly at the dose of about 100 mg twice a day.

In conjunction with the aspects of the present invention, the pharmaceutical compositions comprising (R)-phenylpiracetam may further comprise a carrier or excipient (all pharmaceutically acceptable). The compositions may be formulated e.g. for once-a-day administration, twice-a-day administration, or three times a day administration.

The term "carrier" applied to pharmaceutical compositions of the invention refers to a diluent, excipient, or vehicle with which an active compound (e.g., (R)-
phenylpiracetam) is administered. Such pharmaceutical carriers may be sterile liquids, such as water, saline solutions, aqueous dextrose solutions, aqueous glycerol solutions, and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by A.R. Gennaro, 20th Edition.

[00109] The active ingredient (e.g., (R)-phenylpiracetam) or the composition of the present invention may be used for the treatment of at least one of the mentioned disorders, wherein the treatment is adapted to or appropriately prepared for a specific administration as disclosed herein (e.g., to once-a-day, twice-a-day, or three times a day administration). For this purpose the package leaflet and/or the patient information contains corresponding information.

[00110] The active ingredient (e.g., (R)-phenylpiracetam) or the composition of the present invention may be used for the manufacture of a medicament for the treatment of at least one of the mentioned disorders, wherein the medicament is adapted to or appropriately prepared for a specific administration as disclosed herein (e.g., to once-a-day, twice-a-day, or three times a day administration). For this purpose the package leaflet and/or the patient information contains corresponding information.

[00111] According to the present invention, the dosage form of (R)-phenylpiracetam, or a (R)-phenylpiracetam salt, may be a solid, semisolid, or liquid formulation according to the following.

[00112] (R)-phenylpiracetam may be administered via different application routes. The oral and the parenteral route are the preferred route of application. (R)-phenylpiracetam may be formulated as a flavored liquid, a capsule or a tablet.

[00113] For oral administration in the form of a tablet or capsule, (R)-phenylpiracetam may be combined with non-toxic, pharmaceutically acceptable excipients.
[001 14] The optimal therapeutically effective amount may be determined experimentally, taking into consideration the exact mode of administration, form in which the drug is administered, the indication toward which the administration is directed, the subject involved (e.g., body weight, health, age, sex, etc.), and the preference and experience of the physician or veterinarian in charge.

[001 15] Suitable daily doses of the active ingredient of the invention in therapeutic treatment of humans are within the range from about 1 mg to about 400 mg per day (based on (R)-phenylpiracetam as free base), such as from about 25 mg to about 350 mg, or from about 50 mg to about 300 mg, particularly about 200 mg per day. In an alternative setting, the daily dose may be body weight-adjusted such as about 200 mg/day up to 80 kg body weight or about 240 mg/day for patients with a body weight of ≥ 80 kg. In a further alternative setting, (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof is administered in a range from starting from about 50 mg and increasing the dose in 50 mg steps until the desired therapeutic efficacy is reached, but maximally to about 400 mg/day. Furthermore, in modified release formulations the total amount of active ingredient per day of administration could also be higher due to reduced bioavailability, e.g. up to about 500 mg/day. For use of a pharmaceutically acceptable salt, a solvate, a conjugate or a derivative of (R)-phenylpiracetam, such as (R)-phenylpiracetam hydrochloride, the corresponding amount may be adjusted so that an equimolar amount is used.

[001 16] In certain embodiment, wherein (R)-phenylpiracetam is administered, or where a composition comprising (R)-phenylpiracetam is for administration, with a second agent, the administration may be conjoint or separate.

[001 17] Within the meaning of the present invention, the term "conjoint administration" is used to refer to administration of (R)-phenylpiracetam, and at least one additional active agent simultaneously in different compositions, or sequentially. For the sequential administration to be considered "conjoint", however, (R)-phenylpiracetam, and the at least one additional active agent must be administered separated by a time interval, which still permits the resultant beneficial effect for treating fatigue in a mammal.
EXAMPLES

[001 18] The following example illustrates the invention without limiting its scope.

EXAMPLE 1: Determination of (R)-phenylpiracetam targets

[001 19] Using functional monoamine transporter assays, we determined the potential drug targets for (R)-phenylpiracetam.

[00120] Recombinant Chinese hamster ovary (CHO) cells CHO-K1 (ATCC® CCL-61™) cells stably expressing dopamine transporters are plated. The cells (2 x 10^5/ml) are pre-incubated with test compound and/or vehicle in modified Tris-HEPES buffer pH 7.1 at 25°C for 20 min and 50 nM [3H]-dopamine is then added for an additional 15 min incubation period. Non-specific signal is determined in the presence of 10 μM nomifensine. Cells are then solubilized with 1% SDS lysis buffer. Reduction of [3H]-dopamine uptake by 50 percent or more (>50%) relative to vehicle controls indicates significant inhibitory activity. Compounds are screened at 10, 1, 0.1, 0.01 and 0.001 μM. These same concentrations are concurrently applied to a separate group of untreated cells and evaluated for possible compound-induced cytotoxicity only if significant inhibition of uptake is observed.

[00121] Recombinant Madin Darby canine kidney (MDCK) cells (NBL-2) (ATCC® CCL-34™) expressing norepinephrine transporter are plated for two days. Test compound and/or vehicle is pre-incubated with cells (1 x 10^5/ml) in modified Tris-HEPES buffer pH 7.1 for 20 min at 25°C and 25 nM [3H]-norepinephrine is then added for an additional 15 min incubation period. A lysate is obtained from solubilized cells and counted to determine [3H]-norepinephrine uptake. Reduction of [3H]-norepinephrine uptake by 50 percent or more (>50%) relative to 10 μM desipramine indicates significant inhibitory activity. Compounds are screened at 10, 1, 0.1, 0.01 and 0.001 μM. These same concentrations are concurrently applied to a separate group of untreated cells and evaluated for possible compound-induced cytotoxicity only if significant inhibition of uptake is observed.
[00122] These pharmacological experiments showed an affinity of (R)-phenylpiracetam for the neuronal dopamine re-uptake transporter of 13 µM in functional assays.

[00123] In microdialysis experiments, we could show that at a behaviorally active dose of 100 mg/kg a free concentration 50 µM is reached in extracellular fluid in the brain, and at 50 mg/kg a free concentration of 20 µM.

[00124] These concentrations are sufficiently high to postulate that the dopamine re-uptake transporter is a relevant target in the brain.

**EXAMPLE 2**: Assessment of (R)-phenylpiracetam on extracellular dopamine levels in rat striatum using brain microdialysis - Fig. 1

**Test Item**

[00125] (R)-Phenylpiracetam was dissolved in saline and injected intraperitoneally (i.p).

**Animals**

[00126] Adult male Sprague-Dawley rats (n = 5, 300 g; Harlan, The Netherlands) were used for the experiments. After surgery animals were housed individually (cages 30 cm x 30 cm x 40 cm) with food and water was *ad libitum* available at standard conditions. The post-surgery interval for recovery was minimally 48 h.

**Surgery**

[00127] Rats were anesthetized using isoflurane (2%, 800 ml/min O₂). and placed in a stereotaxic frame (Kopf instruments, USA). L-shaped probes (Hospal AN 69 membrane, 3 mm exposed surface; Brainlink, the Netherlands) were inserted into striatum. Coordinates for the tips of the probes were: posterior (AP) = + 0.9 mm to
bregma, lateral (L) = +3.0 mm to midline and ventral (V) = -6.5 mm to dura (Paxinos and Watson, 1982).

**Microdialysis experiments**

[00128] Experiments were performed 24-48 hours after surgery. On the day of the experiment, the probes were connected with flexible polyether ether ketone (PEEK) tubing to a microperfusion pump (Syringe pump UV 8301501, Univentor, Malta) and perfused with artificial cerebro-spinal fluid (aCSF), containing 147 mM NaCl, 3.0 mM KCl, 1.2 mM CaCl$_2$, and 1.2 mM MgCl$_2$ at a flow rate of 1.5 $\mu$l/min. Microdialysis samples were collected for 20 minute periods up to 120 min using automated fraction collector (Univentor 820 Microsampler, Antec, Netherlands), and stored at -80°C pending analysis. After the experiment, the rats were sacrificed and the brains were removed. The position of each probe was histologically verified according to Paxinos and Watson (1982) by making coronal sections of the brain.

**Determination of Dopamine (DA) and 3,4-Dihydroxyphenylacetic acid (DOPAC)**

**Separation of DA and DOPAC**

[00129] Samples (20 $\mu$l) were injected onto the high-performance liquid chromatography (HPLC) column (Reversed Phase, particle size 3 $\mu$m, C18, Thermo BDS Hypersil column, 150 mm x 2.1 mm, Thermo Scientific, USA) by a refrigerated microsampler system, consisting of a syringe pump (Gilson, model 402, France), a multi-column injector (Gilson, model 233 XL, France), and a temperature regulator (Gilson, model 832, France). Chromatographic separation was performed using a mobile phase that consisted of a NaAc buffer (6.15 g/l) with methyl alcohol (2.5% v/v), Titriplex (250 mg/l), 1-octanesulfonic acid (OSA, 150 mg/l), and adjusted with glacial acetic acid to pH 4.1 (isocratic). The mobile phase was run through the system at a flow rate of 0.35 ml/min by an HPLC pump (Shimadzu, model LC-10AD vp, Japan).
Detection

[00130] Concentrations of DA and DOPAC were determined in the same sample, by HPLC separation and electrochemical detection. DA and DOPAC were detected electrochemically using a potentiostate (Antec Leyden, model Intro, the Netherlands) fitted with a glassy carbon electrode set at +500 mV vs. Ag/AgCl (Antec Leyden, the Netherlands). Data was analyzed by Chromatography Data System (Shimadzu, class-vp, Japan) software. Concentrations were quantified by the external standard method.

Statistical Analysis

[00131] After transmitter levels were stabilized, four consecutive pre-treatment microdialysis samples with less than 50% variation were taken as baseline and their mean was set at 100%. After compound 3-2008 (100 mg/kg) was administered 13 more fractions were taken. Transmitter concentration in each fraction was expressed as % ± SEM (standard error mean) of baseline.

Results

[00132] The results show that (R)-phenylpiracetam increases concentration of dopamine in the striatum.

EXAMPLE 3: Assessment of the concentration of (R)-phenylpiracetam in the brain using brain microdialysis - Figs. 2, 14, 15

Subjects

[00133] Naive adult male Sprague-Dawley rats (240-360 g, Janvier, France) were used for the study kept under standard conditions. All experiments were conducted during the light period of the day-night cycle.

Surgery

[00134] Siliconized guide cannula (MAB 6.14.IC) (MAB, Stockhom, Sweden) were implanted unilaterally in pentobarbital anaesthetized animals aiming at the caudatus
putamen (CPu; AP: +0.1, LM: ± 2.6, DV: -3.2 mm relative to bregma; -3.3 mm interaural) according to the atlas of Paxinos and Watson (loc. cit.). Rats were given at least 3 days to recover from surgery before starting microdialysis experiments.

**Microdialysis**

[00135] Microdialysis experiments were performed in the home cage of the animal. A microdialysis probe (MAB 6.14.4.; 4 mm exposed membrane length, polyethersulfone (PES) membrane; MAB, Stockholm, Sweden) was lowered through the guide cannula into the CPu (ventral position of probe tip with reference to the skull: -7.2 mm) ca. 12 hours before the sampling and left in place for the whole testing period.

[00136] The probes were perfused with aCSF at a flow rate of 2 µl/min using a CMA 102 perfusion pump (CMA, Solna, Sweden). The composition of the aCSF was 147 mM Na⁺, 2.7 mM K⁺, 1.2 mM Ca²⁺, 0.85 mM Mg²⁺, 0.04 mM ascorbic acid. The animals were connected by a head block tether system (Instech, Plymouth Meeting, USA) to a dual channel liquid swivel 375/D/22QM (Instech, Plymouth Meeting, USA). Fluorinated ethylene propylene (FEP) tubing and tubing adapters (MAB, Stockholm, Sweden) were used. The sample collection began one hour after start of perfusion with three 20-minutes fractions (baseline). Thereafter, each rat was injected i.p. with (R)-phenylpiracetam and/or L-DOPA (25 mg/kg + benserazide, 15 mg/kg). The samples (40 µl) were collected automatically with a fraction collector (CMA/142; CMA, Solna, Sweden) and stored at -20°C until analysis.

**Determination of recovery**

[00137] To perform *in vitro* recovery, probes were inserted into a beaker with aCSF (37°C) containing 100 nM solution of (R)-phenylpiracetam or L-DOPA. Five samples (40 µl) were collected while only the last 2 were used for analysis of (R)-phenylpiracetam or L-DOPA concentration.
Analytics

HPLC in combination with atmospheric pressure ionization tandem mass spectrometry (API-MS/MS) was employed (HPLC (Shimadzu Prominance, Duisburg, Germany) coupled to an API 4000 Q Trap (triple quadrupole, Applied Biosystems/MDS Sciex, Darmstadt, Germany) equipped with a Turbolonspray source (ESI)). The analytical column was an Onyx Monolithic C18 50 mm x 2 mm (Phenomenex, Aschaffenburg, Germany). The mobile phase consisted of the eluent A water and eluent B acetonitrile both containing 0.1 % of formic acid. The chromatographic run consisted of a gradient over 1.5 min from 5% acetonitrile in water at start until a mobile phase composition of 50 % acetonitrile in water. A volume of 1 ml was injected in the API/MS/MS. A standard curve was used to calculate sample concentrations of studied agents in our samples.

Results

The results show that (R)-phenylpiracetam reaches brain levels sufficient to affect primary target (DA carrier). Moreover, when injected together with L-DOPA, there is no pharmacokinetic interaction, i.e. (R)-phenylpiracetam does not affect L-DOPA concentration and L-DOPA does not affect (R)-phenylpiracetam concentrations (see Figures 14 and 15). This shows that interactions observed in behavioural experiments are of pharmacodynamic, not pharmacokinetic nature.

EXAMPLE 4: Effect of (R)-phenylpiracetam on locomotor activity (horizontal activity) in rats - Fig. 3

Animals

Experimentally naive adult male Sprague-Dawley rats (230-300 g) are kept four per cage, in a room with controlled temperature (21 ± 1°C) and humidity. Food and water are available ad libitum and the animals are kept under an alternating 12 h / 12 h day-night cycle (lights on at 07.00) for at least 6 days before the experiments are started. Each animal is used only once. Experimental group consist of 8 animals per group.
Procedure

Horizontal locomotor activity was measured in 8 perspex boxes (ENV-51 5-16, 43.2 cm x 43.2 cm x 30 cm), Med-Associates Inc.) equipped with 4 arrays of 16 infrared photobeams placed 3 cm above the floor. Distance travelled (DT) was used in further analysis as a measure of locomotion.

Treatment

(R)-Phenylpiracetam was administered i. p. in a volume of 2 ml/kg in saline

Statistical analysis

Data with locomotor activity were analyzed by means of Kruskal-Wallis ANOVA on ranks followed, if significant, by rank sum test.

Results

Figure 3 shows that (R)-phenylpiracetam increases locomotor activity (horizontal activity) in rats starting at the dose of 100 mg/kg indicating stimulatory activity.

EXAMPLE 5: Testing of (R)-phenylpiracetam in an electroencephalography (EEG) screen - Fig. 4

(R)-Phenylpiracetam was tested at four different concentrations (1 mg/kg, 12.5 mg/kg, 25 mg/kg, and 50 mg/kg) in an EEG screen as described by Dimpfel (Dimpfel, Neuropsychobiology, 58 (2008)178-86).

Animals

Eight adult Fisher 344 rats (8 months of age, kept on an inverse light-dark cycle, weight about 400 g, provided by Charles River Laboratories, D-97633, Sulzfeld)
were used in this experimental series. Animals were implanted with electrodes into the brain and were given two weeks for recovery from surgery (for details see 6.4). After this the transmitter was plugged in for adaptation and control experiments. During the recording rats were not restricted and could move freely but did not have food available (chewing would have produced too many artifacts).

**Acclimatisation and housing conditions**

[00147] The animals were allowed to acclimatize for at least 4 weeks before the study started. There was automatic control of light cycle, temperature and humidity. Light hours were 18 h in the evening - 6 h in the morning. Daily monitoring indicated that temperature and humidity remained within the target ranges of 22 ± 2°C and 44 ± 5% respectively. Cages, bedding, and water bottles were changed at regular intervals, *i.e.* every 2-3 days. Standard diet (Nohrlin H10, Altromin, D-32791 Lage, Germany) was available to the animals *ad libitum*. The animals had access to domestic quality mains water *ad libitum*.

**Surgery**

[00148] Rats were implanted with 4 bipolar concentric steel electrodes within a stereotactic surgical procedure during anaesthesia with ketamine. All four electrodes were placed 3 mm lateral within the left hemisphere. Dorsoventral coordinates were 4, 6, 4.2 and 8 mm and anterior coordinates were 3.7, 9.7, 5.7 and 12.2 mm for frontal cortex, striatum, hippocampus, and reticular formation, respectively (according to the atlas of Paxinos and Watson, 1982). A pre-constructed base plate carrying 4 bipolar stainless steel semi-micro electrodes (neurological electrodes "SNF 100" from Rhodes Medical Instruments, Inc., Summerland, CA 93067, USA) and a 5-pin-plug was fixed to the skull by dental cement interacting with 3 steel screws placed on distance into the bone. The distant recording spot of the electrode was the active electrode whereas the proximal spots of the four electrodes were connected to each other to give a reference. The base plate was carrying a plug to receive later on the transmitter (weight: 5.2 g including battery, 26 mm x 12 mm x 6 mm of size).
Experimental Procedure

EEG signals were recorded from frontal cortex, hippocampus, striatum and reticular formation of freely moving rats from inside a totally copper shielded room. Signals were wirelessly transmitted by a radio-telemetric system (Rhema Labortechnik, Hofheim, Germany, using 40 MHz as carrier frequency) and were amplified and processed as described earlier to give power spectra of 0.25 Hz resolution (Dimpfel et al. 1986; Dimpfel et al. 1988; Dimpfel et al. 1989; Dimpfel, 2003). In short, after automatic artifact rejection signals were collected in sweeps of 4 s duration and fast-fourier transformed using a Hanning window. Sampling frequency was 512 Hz. Four values were averaged to give a final sampling frequency of 128 Hz, well above the Nyquist frequency. The resulting electrical power spectra were divided into 6 specially defined frequency ranges (delta: 0.8 - 4.5 Hz; theta: 4.75 - 6.75 Hz; alpha1: 7.00 - 9.50 Hz; alpha2: 9.75 - 12.50 Hz; beta1: 12.75 - 18.50 Hz; beta2: 18.75 - 35.00 Hz). Spectra were averaged in steps of 3 min each and displayed on-line. In an off-line procedure spectra were averaged to give longer periods for further analysis and data presentation.

Test Items

(R)-Phenylpiracetam was injected i.p. in saline followed by recording of "Tele-Stereo-EEG" intracerebral field potentials in combination with a video tracking system for detection of changes in motility (GJB Datentechnik GmbH, D-98704 Langewiesen, Germany). This system recognized locomotion as well as stereotyped behaviour by following a contrast difference of the black transmitter on the head of the animal in comparison to its environment. The system has been validated in a previous study with different dosages of caffeine.

Treatment Groups

After surgery all animals were randomly allocated to treatment groups, such that the treatment groups were evenly distributed throughout the caging system. A crossover design with at least one week of drug holidays in between the administrations was used. After a pre-drug period of 45 min for pre-drug recording, drug effects were observed continuously on the screen (artifact control) for 300 min subdivided into 15
min periods after a lag time of 5 min for calming of animals after i.p. administration. Changes of electrical power are expressed as % of the 45 min absolute pre-drug spectral power values within each frequency band.

Statistical Analysis

[00152] Data are expressed as mean values ± S.E.M. Statistics were calculated by means of the Wilcoxon-Mann-Whitney U-test for comparison to results obtained by vehicle injection at the particular time frame. For comparison of data to reference compounds tested earlier under identical conditions discriminant analysis according to Fischer was used. A total of 24 variables (six frequency ranges times 4 brain areas) were used for analysis. Firstly, spherical projection of the results from 47 reference compounds plus physiological sleep was performed using the three spatial coordinates for the first three discriminant axes. Secondly, coding of the result of the fourth to sixth discriminant analysis into red, green and blue was followed by an additive color mixture in analogy to the so-called RGB mode (as used in TV). This matrix of drug actions is kept constant (frozen) for classification of unknown preparations since addition of further compounds would otherwise change the projections.

Results

[00153] (R)-Phenylpiracetam produced a dose dependent attenuation of alpha2 and betal waves. The highest dosage produced a different pattern of changes in that theta power increases within the frontal cortex were observed. Motility was increased. Alpha2 waves are mainly under the control of dopamine (Dimpfel, loc. cit.). Thus, direct or indirect effects on dopaminergic neurotransmission can be expected from (R)-phenylpiracetam. Attenuation of alpha2 and betal waves has also been observed after administration drugs used for treatment of M. Parkinson (Dimpfel and Hoffmann, Neuropsychobiology, 62, 213-20, 2010). In summary (R)-phenylpiracetam shows a profile within the area of stimulatory drugs.
EXAMPLE 6: Effect of (R)-phenylpiracetam in motivation tests - Figs. 5, 6, 7, 8

Test Item
[00154] Methylphenidate purchased from Sigma (Taufkirchen, Germany) was dissolved in distilled water fresh for each test day. Modafinil purchased from Sequoia Research Products Limited (Pangbourne, UK) was dissolved in 1% w/v methylcellulose (Sigma, Taufkirchen, Germany) in 0.9% NaCl water fresh for each test day. (R)-Phenylpiracetam was dissolved in distilled water fresh for each test day.

Animals
[00155] One hundred and three male Sprague-Dawley rats (Janvier, Le Genest-St-Isl, France) weighing 225-250 g on arrival were used.

Acclimatisation and housing conditions
[00156] The animals were kept under standard laboratory conditions in groups of up to 4 per care with ad libitum access to water. Standard diet for rodents (Altromin) was available to the animals at 15 g per animal/day

Treatment Groups
[00157] Two experiments were performed using an identical protocol. After continuous reinforcement training (CRF) training (see below) all animals were assigned to treatment groups, such that performance across all the groups was similar. The treatment groups were evenly distributed throughout the caging system.

Experimental methods
[00158] In experiment 1 and 2 the following protocols were used:
[00159] Overview: Three tests were performed in a consecutive manner on subsequent days. On day 1-2, animals were habituated individually to Skinner boxes in two sessions, one per day, for 30 min. Animals received "free" food pellets at a random time (one pellet per minute on average). On days 3-8 animals were trained in Skinner
boxes on a continuous reinforcement schedule (one pellet per lever press). On days 9-10 they were tested in the progressive ratio test (Test 1) off-drug, on days 11-12 on-drug. On day 13, a choice test (Test 2) was performed on-drug, on day 14, a consumption test (Test 3) was performed on-drug. Below, tests are described in detail.

**Test 1: Progressive ratio (PR) task**

[00160] Behavioral testing was conducted in 12 operant test chambers (Med Associates, St. Albans, USA). Each chamber was equipped with a retractable lever, a food dispenser with receptacle, an overhead house light and two stimulus lights, one above the lever and the other above the food receptacle. An infrared photocell beam detected nose pokes into the food receptacle. The apparatus was controlled by a computer system (SmartControl®-Interface and MedPC-software, Med Associates, St. Albans, USA). The light above the food receptacle indicated the delivery of a food pellet in the receptacle. Rats were first habituated to the operant boxes for two sessions (30 min each) on two consecutive days. Thereafter, animals were trained for six sessions on the CRF schedule for 30 min. Followed by 4 sessions (two session off-drug followed by two sessions on-drug) of an increasing fixed ratio (FR) schedule in steps of 5, with 3 repetitions of each step (*i.e.* 1-1-1; 5-5-5; 10-10-10; *...*). When the required FR value of each trial was achieved the light above the food receptacle indicated the delivery of a single food pellet (45 mg, Bioserve, USA). The light remained on until the rat poked its nose in the receptacle. Lever presses while the light was on were counted as perseverative lever presses; they were counted but had no programmed consequences. A session lasted for 90 min or was ended when a rat failed to press the lever for consecutive 10 min. For each session, the value of the last completed ratio (breaking point) was recorded as well as the amount of received rewards, the perseverative lever presses, the duration of the session and the latency to respond. Animals of all treatment groups received respective vehicle/drug infusions 30 min pre-test on the final two sessions involving testing under a PR schedule.

**Test 2: Cost-benefit choice test**

[00161] In this task the rats had the choice between working for their preferred food (Bioserve pellets) by pressing the lever on a PR schedule as described above or
obtaining lab chow being freely available in a dish (about 15 g) within the operant chamber. The food receptacle and the lever were positioned on the same wall of the operant chamber, the dish containing the lab chow was situated in a corner on the opposite side of the operant chamber. A session lasted for 90 min or ended when a rat failed to press the lever for consecutive 10 min. Thereafter the amount of lab chow ingested was calculated. Animals of all treatment groups received vehicle/drug infusions 30 min pre-test.

**Test 3: Consumption test**

[00162] The animals were placed individually in separate cages (type III) containing a glass bowls filled with 30 g of pellets. The animals had free access to the reward for 20 min and the amount consumed was measured by subtracting the weight of each glass bowl before the 20 min consumption test and the weight of each glass bowl after the test. Animals of all treatment groups received vehicle/drug infusions 30 min pre-test.

**Statistical Analysis**

[00163] The data were subjected to one or two way ANOVAs followed by Dunnett’s post hoc test. All statistical computations were carried out with STATISTICS™ (StatSoft®, Tulsa, USA). The level of statistical significance (a-level) was set at p < 0.05.

**Results**

[00164] Present data show that (R)-phenylpiracetam increases motivation, *i.e.*, the work load, which animals are willing to perform to obtain more rewarding food. At the same time consumption of freely available normal food does not increase. Generally this indicates that (R)-phenylpiracetam increase motivation and in turn effect on mental fatigue would be expected. The effect of (R)-phenylpiracetam is much stronger than that of methylphenidate and amphetamine. Moreover, (S)-phenylpiracetam produces only a very weak effect at 100 mg/kg, which is 2.5 times lower than (R)-phenylpiracetam, and no significant effect at 200 mg/kg.
EXAMPLE 7: Effect of (R)-phenylpiracetam on rats with unilateral SNc lesion
(model of Parkinson’s Disease) - Figs. 9, 10

[00165] (R)-Phenylpiracetam was tested in rats with unilateral SNc lesions - a preclinical model of Parkinson’s disease. In some experiments it was combined with L-DOPA. In this model, ipsilateral rotations indicate a presynaptic mode of action, which is consistent with inhibition of dopamine uptake as a primary mode of action. L-DOPA produced contralateral rotations.

Animals

[00166] Male Sprague-Dawley rats that have undergone unilateral lesion of medial forebrain bundle using 6-hydroxydopamine and showed a clear-cut ipsilateral rotation bias in amphetamine rotation test. The rats were housed four per cage in the animal room with a controlled 12-hour light-dark cycle and controlled temperature (21 °C) with access to standard laboratory food (chow pellets) and tap water ad libitum. All experiments were carried out between 09:00 and 16:00.

Chemicals

[00167] (R)-Phenylpiracetam was injected i.p. in saline. L-DOPA (25 mg/kg) and benserazide (15 mg/kg) were dissolved in saline and injected i.p.

Rotation test

[00168] Rats were injected with substance, placed in Perspex cylinders (30 cm diameter), and the rotational behaviour (360°) was scored for 120 min at 20-min intervals using TSA rotation measurement system.
Statistical analysis

[00169] Data are presented as means +/- SEM. Sums of total scores obtained during a whole recorded period was analysed using two-way ANOVA. If significant, the two-way ANOVA was followed by the Dunnett test for pair-wise multiple comparisons.

Results

[00170] This study showed that (R)-phenylpiracetam increased ipsilateral rotations in a PD animal model. These results suggest that (R)-phenylpiracetam improves fatigue associated with Parkinson's disease and may have also antiparkinsonian activity. In summary, these results suggest that (R)-phenylpiracetam fulfils the criteria of an effective and well-tolerated treatment for PD patients suffering from fatigue and fatigue-associated symptoms, like inactivity, motivational-deficit, apathy, floppiness, exhaustion, lassitude, prostration etc.

[00171] Based on these initial findings, we can conclude that (R)-phenylpiracetam is a candidate for the treatment of fatigue associated with PD due to its effect on the dopamine re-uptake transporter.

EXAMPLE 8: Effect of (R)-phenylpiracetam on L-DOPA-induced dyskinesia - Fig. 11

Animals

[00172] Male Sprague-Dawley rats that underwent stereotaxic lesions of SNc and VTA and reached scores of at least 5.0 net right turns in amphetamine rotation test should be used in this procedure. The animals were housed in a standard laboratory conditions.

Chemicals

[00173] 3,4-Dihydroxy-L-phenylalanine methyl ester (L-DOPA) and benserazide hydrochloride (benserazide) were dissolved in saline (1 ml per 1 kg rat) and applied simultaneously, i.p., at doses of 6 and 15 mg/kg, respectively. The rats were treated
with L-DOPA/benserazide daily, for 19-21 consecutive days until dyskinesia developed. (R)-Phenylpiracetam was injected i.p. in saline.

**Rating of abnormal involuntary movements (AIMs)**

[00174] Rating of dyskinetic-like movements such as locomotive dyskinesia, limb dyskinesia, axial dystonia, orolingual dyskinesia was scored as described previously and total score was used for the analysis (Dekundy et al., Pharmacological characterization of MRZ-8676, a novel negative allosteric modulator of subtype 5 metabotropic glutamate receptors (mGluR5): focus on L:-DOPA-induced dyskinesia. J Neural Transm 118, 1703-1716, 2011).

**Results**

[00175] The data show that (R)-phenylpiracetam does not enhance dyskinesia produced after L-DOPA at the doses, which enhance antiparkinsonian effects of L-DOPA.

**EXAMPLE 9: Effect of (R)-phenylpiracetam on sedation induced by reserpine**

**Fig. 12**

**Animals**

[00176] Male Sprague-Dawley rats weighing ca. 300 g were kept under standard laboratory conditions.

**Chemicals**

[00177] Animals were injected with reserpine (5 mg/kg) and a-MT (250 mg/kg) 24 and 3.5 h before testing, respectively. (R)-phenylpiracetam (25, 50 or 100 mg/kg) was injected i.p directly before the test.
**Locomotor activity in reserpine-treated rats**

The locomotor activity was measured in four perspex boxes (ENV-515-16, 43.2 cm x 43.2 cm x 30 cm), Med-Associates Inc. system) equipped with 4 arrays of 16 infrared photobeams placed 3 cm above the box floor. Distance travelled (DT) was used in further analysis for measuring locomotion. The recording started immediately after placing animals in the open field.

**Statistical analysis**

Sums of total scores obtained during a whole recorded period was analysed by one-way ANOVA.

**Results**

The data show that (R)-phenylpiracetam attenuates hypokinesia produced by reserpine supporting anti-fatigue and antiparkinsonian activity.

**EXAMPLE 10: Effect of (R)-phenylpiracetam on sedation induced by haloperidol - Fig. 13**

**Animals**

Male Sprague-Dawley rats weighing ca. 300 g were kept under standard laboratory conditions.

**Chemicals**

(R)-Phenylpiracetam (25, 50 and 100 mg/kg) was injected i.p. in saline directly before the test. Haloperidol was also given i.p. at the dose of 0.2 mg/kg, 30 min before the test start.
**Locomotor activity in haloperidol-treated rats**

The locomotor activity was measured in four perspex boxes (ENV-515-16, 43.2 cm x 43.2 cm x 30 cm), Med-Associates Inc. system) equipped with 4 arrays of 16 infrared photobeams placed 3 cm above the box floor. Distance travelled (DT) was used in further analysis for measuring locomotion. The recording started immediately after placing animals in the open field.

**Statistical analysis**

Sums of total scores obtained during a whole recorded period were analysed by one-way ANOVA, followed, when significant, by the Holm-Sidak test.

**Results**

The data show that (R)-phenylpiracetam dose-dependently attenuates hypokinesia produced by haloperidol supporting anti-fatigue and antiparkinsonian activity (Fig. 13). An additional experiment shows that (S)-phenylpiracetam is much less potent as it produces no effect at 50 mg/kg (Fig. 16A) or an almost negligible effect at 100 mg/kg (Fig. 16B). It should be noted that (R)-phenylpiracetam produced a very robust effect at these doses.

**References**


* * * * *

[00186] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

[00187] To the extent possible under the respective patent law, all patents, applications, publications, test methods, literature, and other materials cited herein are hereby incorporated by reference.
CLAIMS

1. A pharmaceutical composition comprising (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof for use in combination treatment with an agent for dopamine-replacement therapy of Parkinson's disease.

2. The pharmaceutical composition of claim 1, wherein said agent is L-DOPA.

3. The pharmaceutical composition according to claim 1 or 2, wherein the amount of said agent used in said combination treatment is reduced compared to the amount administered in the treatment cycle prior to said combination treatment.

4. The pharmaceutical composition according to claim 1 or 2, wherein said agent is administered in said combination treatment at a reduced frequency compared to the frequency of administration in the treatment cycle prior to said combination treatment.

5. The pharmaceutical composition according to claim 4, wherein said agent is L-DOPA, which is administered once daily.

6. The pharmaceutical composition according to claim 1 or 2, wherein (R)-phenylpiracetam is for use in a treatment comprising the following steps:

   a. administering (R)-phenylpiracetam to a patient under dopamine-replacement therapy in combination with said agent administered for dopamine-replacement;

   b. reduction of said agent in daily increments of 10% until signs of Parkinson's symptoms re-emerge; and

   c. increase of said agent once by 10% compared to the amount of said agent last administered according to step b.; and

   d. continuation of the combination treatment using (R)-phenylpiracetam and the amount of said agent used in accordance with step c.
7. The pharmaceutical composition according to claim 6, wherein said steps are performed in order to establish said combination treatment.

8. The pharmaceutical composition according to claim 6, wherein said steps are performed in order to improve an established combination treatment.


10. The pharmaceutical composition according to any one of claims 1 to 9, wherein (R)-phenylpiracetam is present as (R)-phenylpiracetam hydrochloride.

11. The pharmaceutical composition according to any one of claims 1 to 10, wherein (R)-phenylpiracetam is for administration in a range from about 1 mg to about 250 mg/day, or in a range from about 25 mg to about 200 mg/day, or in a range from about 50 mg to about 150 mg/day.

12. The pharmaceutical composition according to any of the preceding claims, wherein (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof is for administration once a day, twice a day, or three times a day.

13. The pharmaceutical composition according to any of the preceding claims, wherein (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof is for administration in an oral formulation.

14. The pharmaceutical composition according to any of the preceding claims, wherein (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof is for administration in combination with at least one additional pharmaceutical agent which has been shown to be effective for the treatment of Parkinson's disease.

15. The pharmaceutical composition according to claim 14, wherein said at least one additional pharmaceutical agent is selected from: L-DOPA, carbidopa, other types of dopamine agonists, including eltoprazine, MAO-inhibitors, COMT inhibitors, and benserazide.
16. The pharmaceutical composition according to any of the preceding claims, wherein (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof is for administration in combination with at least one additional pharmaceutical agent which has been shown to be effective for the treatment of fatigue, particularly mental fatigue, associated with Parkinson’s disease.

17. The pharmaceutical composition according to claim 16, wherein said at least one additional pharmaceutical agent is selected from: rasagiline and pramipexole.

18. A method of improving the dopamine-replacement therapy of Parkinson’s disease and/or of treating fatigue, particularly mental fatigue, associated with Parkinson’s disease in a subject in need thereof, comprising the step of administering an effective amount of (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof.

19. The method of claim 18, wherein said agent is L-DOPA.

20. The method according to claim 18 or 19, wherein the amount of said agent used in said combination treatment is reduced compared to the amount administered in the treatment cycle prior to said combination treatment.

21. The method according to claim 18 or 19, wherein said agent is administered in said combination treatment at a reduced frequency compared to the frequency of administration in the treatment cycle prior to said combination treatment.

22. The method according to claim 21, wherein said agent is L-DOPA, which is administered once daily.

23. The method according to claim 18 or 19, wherein (R)-phenylpiracetam is for use in a treatment comprising the following steps:

   a. administering (R)-phenylpiracetam to a patient under dopamine-replacement therapy in combination with said agent administered for dopamine-replacement;

   b. reduction of said agent in daily increments of 10% until signs of Parkinson’s symptoms re-emerge; and
c. increase of said agent once by 10% compared to the amount of said agent last administered according to step b.; and

d. continuation of the combination treatment using (R)-phenylpiracetam and the amount of said agent used in accordance with step c.

24. The method according to claim 23, wherein said steps are performed in order to establish said combination treatment.

25. The method according to claim 23, wherein said steps are performed in order to improve an established combination treatment.

26. A method of treating fatigue associated with Parkinson's disease in a subject in need thereof, comprising the step of administering an effective amount of (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof.

27. The method according to any one of claims 18 to 26, wherein (R)-phenylpiracetam is present as (R)-phenylpiracetam hydrochloride.

28. The method according to any one of claims 18 to 27, wherein (R)-phenylpiracetam is for administration in a range from about 1 mg to about 250 mg/day, or in a range from about 25 mg to about 200 mg/day, or in a range from about 50 mg to about 150 mg/day.

29. The method according to any of the preceding claims, wherein (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof is for administration once a day, twice a day, or three times a day.

30. The method according to any of the preceding claims, wherein (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof is for administration in an oral formulation.

31. The method according to any of the preceding claims, wherein (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof is for administration in combination with at least one additional pharmaceutical agent which has been shown to be effective for the treatment of Parkinson's disease.
32. The method according to claim 31, wherein said at least one additional pharmaceutical agent is selected from: L-DOPA, carbidopa, other types of dopamine agonists, including eltoprazine, MAO-inhibitors, COMT inhibitors, and benserazide.

33. The method according to any of the preceding claims, wherein (R)-phenylpiracetam or a pharmaceutically acceptable salt thereof is for administration in combination with at least one additional pharmaceutical agent which has been shown to be effective for the treatment of fatigue, particularly mental fatigue, associated with Parkinson’s disease.

34. The method according to claim 33, wherein said at least one additional pharmaceutical agent is selected from: rasagiline and pramipexole.
Figure 1

The graph illustrates the changes in DA levels (% of baseline) over time (min) for different doses of (R)-phenylpiracetam. The lines represent:
- Open circles: (R)-phenylpiracetam (10 mg/kg)
- Solid circles: (R)-phenylpiracetam (50 mg/kg)
- Solid triangles: (R)-phenylpiracetam (100 mg/kg)

The x-axis shows time in minutes, ranging from -60 to 300, while the y-axis shows DA levels in % of baseline, ranging from 80 to 150.

The graph shows fluctuations in DA levels with time, with the highest levels occurring around the 120-130% range. The different doses show varying degrees of effect, with the 100 mg/kg dose having the most pronounced effect.
Figure 2

Concentration of (R)-phenylpiracetam in the brain after i.p. application as assessed using brain microdialysis

- (R)-phenylpiracetam (10 mg/kg)
- (R)-phenylpiracetam (50 mg/kg)
- (R)-phenylpiracetam (100 mg/kg)

Administration

Corrected for in vitro recovery
Figure 3

Effect of (R)-phenylpiracetam on locomotor activity in rats (horizontal activity)

*P<0.05 vs vehicle and (R)-phenylpiracetam 10 mg/kg; Kruskal-Wallis one-way ANOVA on ranks at each time interval followed by rank sum test

(R)-phenylpiracetam was administered i.p., 15 min. before test.
Figure 4

65 - 125 min after administration

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Figure 5

Progressive Ration test Measuring Motivation

- ● Vehicle
- ○ Amphetamine (1 mg/kg)
- ▼ (R)-phenylpiracetam (25 mg/kg)
- △ (R)-phenylpiracetam (50 mg/kg)
- ■ (R)-phenylpiracetam (100 mg/kg)

Maximum # lever presses

0 50 100 150 200

drug-free  on drug

Days

1 2 3 4
Figure 6

Progressive Ratio Test Measuring Motivation

- Vehicle
- Methylphenidate 5 mg/kg
- Methylphenidate 10 mg/kg
- (R)-phenylpiracetam 50 mg/kg
- (R)-phenylpiracetam 100 mg/kg

Maximum lever presses (number)

0  50  100  150  200

drug-free  on drug

1  2  3  4  Days

*
Figure 7

Choice test:
Lever presses for preferred pellets

Intake of free, less preferred lab chow

Maximum # lever presses (+/-SEM)

Vehicle
Amphetamine (1 mg/kg)
(R)-phenylpiracetam (25 mg/kg)
(R)-phenylpiracetam (50 mg/kg)
(R)-phenylpiracetam (100 mg/kg)

Amount ingested (g)

*
Figure 8

Choice test: lever presses for preferred food

Intake of free, less preferred lab chow

Maximum lever presses (number)

Amount ingested (g)

- Vehicle
- Methylphenidate (5 mg/kg)
- Methylphenidate (10 mg/kg)
- (R)-phenylpiracetam (50 mg/kg)
- (R)-phenylpiracetam (100 mg/kg)
Figure 9

Effect of (R)-phenylpiracetam on rotations in hemiparkinsonian rats

Contralateral rotations (number, 180 min)

(R)-phenylpiracetam (mg/kg)
Effect of (R)-phenylpiracetam on turning behaviour in 6-OHDA lesioned rats

- O: vehicle - vehicle
- ▪: L-DOPA (25 mg/kg) - vehicle
- ▲: vehicle - (R)-phenylpiracetam (50 mg/kg)
- ▼: L-DOPA - (R)-phenylpiracetam

* p<0.05 vs vehicle, # p<0.05 vs L-DOPA - saline; two-way RM ANOVA followed by Tukey's test
Effect of (R)-phenylpiracetam on L-DOPA-induced dyskinesia in 6-OHDA-lesioned hemiparkinsonian rats - time course
Figure 12

Effect of (R)-phenylpiracetam on reserpine-induced hypolocomotion in rats (horizontal activity)

Distance travelled (cm: 120 min)

vehicle 25 50 100
(R)-phenylpiracetam (mg/kg)

*
Effect of (R)-phenylpiracetam on haloperidol-induced hypolocomotion in rats (horizontal activity)

*\( P < 0.05 \) vs vehicle; one-way ANOVA followed by Duncan’s test
Concentration of L-Dopa in combination with (R)-phenylpiracetam in the brain after i.p. application as assessed using brain microdialysis

Corrected for in vitro recovery
Figure 15

Concentration of (R)-phenylpiracetam in combination with L-Dopa in the brain after i.p. application as assessed using brain microdialysis

Corrected for in vitro recovery
(R)-phenylpiracetam and L-Dopa were administered in saline
Figure 16:

A:

Effect of phenylpiracetam on haloperidol induced hypolocomotor activity in rats (horizontal activity)

*P < 0.05 vs other groups; one-way ANOVA followed by Duncans test

B:

Effect of phenylpiracetam on haloperidol induced hypolocomotor activity in rats (horizontal activity)

*P < 0.05 vs vehicle; one-way ANOVA followed by Duncans test

#P < 0.05 vs (S)-phenylpiracetam; one-way ANOVA followed by Duncans test
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

A61K31/496

ADD.

According to International Patent Classification (IPC) or both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, SCISEARCH, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Date of the actual completion of the international search 17 September 2013

Date of mailing of the international search report 24/09/2013

Name and mailing address of the ISA
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Authorized officer Horni ch-Paraf, E

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