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A Randomized, Double-Blind, Placebo-Controlled, Crossover Study to Evaluate the Safety and Efficacy of the 5-Hydroxytryptophan on REM Sleep Behavior Disorder, Levodopa-Induced Motor Complications and Neuropsychiatric Disorders in Idiopathic Parkinson's disease.

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Presentata da: Mario Meloni

Coordinatore Dottorato   Prof. Antonio Argiolas

Tutor: Dott.ssa Monica Puligheddu

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Abstract

Background and purpose: Parkinson's disease (PD) is a chronic neurodegenerative disorder characterized by the motor symptoms of bradykinesia, tremor, rigidity and postural instability. However, non-motor symptoms such as depression, apathy and rapid eye movement sleep behavior disorder (RBD) are also frequent and play a significant role. Several studies have indicated that altered serotonergic neurotransmission may contribute to the motor and non-motor features commonly associated with PD such as levodopa-induced motor complications, sleep disorders, apathy and depression.The 5-Hydroxytryptophan (5-HTP) is the intermediate metabolite of L-tryptophan in the production of serotonin. Studies in other populations indicate that 5-HTP might be useful in the treatment of depression, chronic headache and sleep disturbances. Furthermore, a recent study demonstrated the antidyskinetic effect of 5-HTP in the rat model. To date, there has been inconsistent research on the use of 5-HTP in PD. The purpose of this study was to compare the effects of 5-HTP to placebo on RBD, depression, apathy and levodopa-induced motor complications in patients with PD.

Materials and Methods: A single-center, randomized, double-blind placebo-controlled cross-over trial was employed; 36 subjects were subsequently enrolled into the study, 32 subjects completed the 16-week protocol. Patients received placebo and 50 mg of 5-HTP daily in a cross-over design over a period of 4 weeks. There were 4-weeks washouts between treatments. For the assessment of efficacy on the RBD, video-polysomnography (v-PSG) was performed at baseline, and home-based polysomnography was performed at weeks 4 and 12. For the assessment of efficacy on the overall functional status, depression, apathy and motor complications, the UPDRS (parts I, II, III), Beck Depression Inventory II (BDI-II), 21-item version of Hamilton Depression Rating Scale ($HDRS_{21}$), Apathy Scale (AS), Unified Dyskinesia Rating Scale (UDyRS), UPDRS IV, Wearing-Off Questionnaire (WOQ-19) and the self-reported 24-hour home dyskinesia diaries were respectively obtained at baseline and weeks 4, 8, 12 and 16 (T-end).

Primary efficacy outcomes were the effect of 5-HTP on the the percentage of REM Sleep without Atonia (RSWA) and other polysomnographic sleep measures compared to placebo as well as the comparison of 5-HTP to placebo in mean change from baseline to weeks 4, 8, 12 and 16 in total score on the AS, BDI-II, $HDRS_{21}$, UDysRS, UPDRS IV and WOQ-19.

Results: Repeated measures analysis revealed a significant improvement of levodopa-induced dyskinesias (LIDs) and depressive symptoms during the 50 mg 5-HTP treatment compared to placebo as assessed by the UDyRS, UPDRS-IV and $HDRS_{21}$. Treatment with 5-HTP significantly reduced the percentage of time in stage N3 compared to placebo.

Furthermore, the 5-HTP produced a marginal, non-significant reduction in arousal index, wake after sleep onset (WASO) as well as an increase in total percentage of REM sleep. There were no other significant effects of 5-HTP at dose 50 mg compared to placebo on nighttime sleep parameters evaluated in our study.

Conclusions: This study provides preliminary evidence of clinical benefit with 5-HTP for treating LIDs and depressive symptoms in PD. Larger studies with a longer treatment duration need to corroborate these early findings.

Chapter 1: Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder and is associated with progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc). By the time of death, this region of the brain has lost 50-70% of its neurones compared with the same region in unaffected individuals. The resultant dopamine deficiency within the basal ganglia leads to a movement disorder characterised by classical parkinsonian motor symptoms. Previously, PD was thought to be caused primarily by environmental factors, but research is revealing that the disease develops from a complicated interplay of genetics and environment. Thus, PD is now viewed as a slowly progressive neurodegenerative disorder that begins years before diagnosis can be made, implicates multiple neuroanatomical areas, results from a combination of genetic and environmental factors, and manifests with a broad range of symptoms. The gold standard for diagnosis of PD has been the presence of SNpc degeneration and Lewy pathology at post-mortem pathological examination. Lewy pathology consists of abnormal aggregates of α-synuclein protein, called Lewy bodies and Lewy neurites. They are a-synuclein-immunoreactive inclusions made up of a number of neurofilament proteins together with proteins responsible for proteolysis. These include ubiquitin, a heat shock protein which plays a key role in targeting other proteins for breakdown. The association between Lewy pathology and pathogenesis of the disease is still poorly understood.

The diagnosis of PD remains essentially a clinical one, and it is important to recognize the early features together with symptoms and signs suggesting other causes of parkinsonism.

Central physical features include resting tremor, bradykinesia, rigidity and postural instability [1].

The mainstay of PD management is symptomatic treatment with drugs that increase dopamine concentrations or directly stimulate dopamine receptors. However, PD involves neurotransmitters other than dopamine and regions of the nervous system outside the basal ganglia. Non-motor features including cognitive and neuropsychiatric disturbances also commonly manifest, dispelling previous beliefs that PD is solely a disorder of movement [2]. Indeed, contrary to previous perceptions, PD is recognised as a multisystem disorder. The pathological processes in PD are not only confined within the dopaminergic system as there is a more diffuse pathology involving other non-dopaminergic systems. Besides dopamine (DA), three further key neurotransmitters have been described to be involved in the pathogenesis of PD; namely noradrenaline (NA), acetylcholine (ACh), and serotonin (5HT) [3;4].

Consequentially, non-motor symptoms in PD can potentially be related to dopaminergic, non-dopaminergic pathogenesis or a combination of both [5].

Individual studies indicate that apathy [6], anxiety [7] as well as aspects of sleep disturbances [8] appear to be linked to striatal dopaminergic deficiency as measured by dopamine transporters (DaT) scans. However, non-motor symptoms such as depression [9;10], apathy [10] and rapid eye movement sleep behavior disorder (RBD) [11] may be driven by deficiency in non-dopaminergic transmitters.

In particular, the serotonergic system play a significant role on cognition, emotion and motor behaviour; thus altered serotonergic neurotransmission may contribute to the motor and non-motor features commonly associated with PD such as motor complications, sleep disorders, apathy, depression and psychosis.

Chapter 2: Serotonergic dysfunction in Parkinson's disease

Serotonin (5-hydroxytryptamine, 5-HT) is a bioactive substance synthesized from tryptophan and is widely distributed in most peripheral tissues and the brain [12].

In the brain, serotonin cell bodies are located in the raphe nuclei of the brainstem (pons and medulla oblongata), where they can be subdivided on the basis of their distribution and main projections into two groups: the rostral group with major projections to the cerebral cortex, limbic structures (e.g., hippocampus and amygdala), basal ganglia (e.g., striatum), diencephalon (e.g., thalamus and hypothalamus) [13;14] and the caudal group with major projections to the caudal brainstem and to the spinal cord [15].

The serotonergic neurotransmission is mediated by 5-HT receptors that are generally classified into 7 families (5-HT1 to 5-HT7) according to the signal cascades, encompassing at least 14 subtypes (5-HT1A, 1B, 1D, 1E, 1F, 5-HT2A, 2B, 2C, 5-HT3, 5-HT4, 5-HT5A, 5B, 5-HT6 and 5-HT7) [16]. These 5-HT receptors, except for 5-HT3 receptors, are G protein-coupled receptors with seven transmembrane spanning domains. They are distributed in the post-synaptic membranes of neurons or nerve terminals innervated by 5-HT neurons and mediate intracellular signal transduction via coupling to individual G proteins. On the other hand, 5-HT3 receptors are composed of a hetero-pentamer consisting of 5 subunits, 5-HT3A to 5-HT3E, which forms Na^+ and Ca^{2+} permeable cation channels [17]. In addition to the post-synaptic 5-HT receptors, 5-HT neurons have 2 types of pre-synaptic autoreceptors, 5-HT1A and 5-HT1B/1D receptors. 5-HT1A receptors are located on the cell bodies of 5-HT neurons and negatively control their own firing, while 5-HT1B/1D receptors exist on the nerve terminals of 5-HT neurons and inhibit the release or synthesis of 5-HT as a feedback mechanism [18].

Progress in 5-HT research has led to the development of various therapeutic agents which selectively interact with 5-HT receptors or transporters such as the selective 5-HT reuptake inhibitors, the 5-HT1A agonistic anxiolytics and the second generation antipsychotics with potent 5-HT2 blocking actions [19;20].

These serotonergic drugs have being greatly contributed to the current medications for various chronic neuropsychiatric diseases including PD.

Although the dopaminergic system has long been considered to be the primary cause of PD, several lines of evidence revealed that the serotonergic system also plays important roles in modulating extrapyramidal motor functions and other non-motor symptoms related to PD [21;22].

Evidence from animal, biochemical, post-mortem and human in-vivo studies have demonstrated loss of striatal and extra-striatal serotonin markers in the course of PD indicating that the serotonergic system is affected from PD pathology [23;24;25].

According to Braak's staging, the pathological process in PD occurs in a gradual ascending fashion, starting from the olfactory nucleus and the medulla in presymptomatic stages and spreading to the pons and midbrain later. In 'Braak' stage two, Lewy body and Lewy neurite deposition occurs within the median raphe nuclei containing the serotonergic neurons of the caudal brainstem [26;27]. These data suggest that caudal brainstem serotonergic neurons are affected before dopaminergic midbrain neurons while in the midbrain the two systems should be affected simultaneously.

5-HT content and the density of 5-HT transporters (a marker for 5-HT nerve terminals) in the forebrain regions (e.g., striatum and neocortex) are also reduced in the advanced stage of PD [28; 29]. Interestingly, the level of striatal 5-HT transporter increased in the early stage of PD, which temporally correlated with a reduction in striatal dopamine transporters, implying an initial compensatory mechanism of the serotonergic system in PD [30].

A recent postmortem study [31] has shown a serotonergic hyperinnervation in the striatum, with the detection of dopamine in serotonergic varicose fibers. These findings may be interpreted as a compensatory mechanism for the lost function of dopaminergic neurons, due to the ability of 5-HT neurons to convert levodopa to dopamine [32].

Yet, the influence exerted by 5-HT impairment in PD remains unclear. However, serotonergic system degeneration in early and late stage of PD seems to be important in generation of motor and non-motor symptoms in patients with PD.

Further, several studies, conducted in rodent or primate's PD models, have focused on the role of different 5-HT receptor subtypes in either motor or non motor disease manifestations with investigational trials on promising 5- HT ligands [33].

2.1 Motor complications

Progression of PD is characterised by worsening of motor features, which initially can be managed with symptomatic therapies such as levodopa and dopamine agonists. However, as the disease advances, there is an emergence of complications related to long-term symptomatic treatment, including motor fluctuations and dyskinesias. Complications of long-term dopaminergic treatment are features of advanced disease. Fluctuations and dyskinesias are believed to result, in part, from pulsatile stimulation of striatal dopamine receptors, which occurs later in the disease when

intracerebral levodopa concentrations become more closely linked to plasma levodopa concentrations [34].

2.1.1 Motor fluctuactions

The long-term use of dopamine replacement therapy demonstrated that with disease progression, motor fluctuations, characterized by "wearing-off" and motor complications in the form of dyskinesia, become increasingly problematic in maintaining symptomatic control [35]. After some years of stable and sustained response to levodopa therapy, the effect of a single levodopa dose become progressively shorter and most patients experience fluctuations in motor performance. Also, periods of immobility unrelated to times of levodopa supply occur in most advanced cases (on-off phenomenon).

Wearing-off (WO) is defined as a gradual decrease in the duration of effect of each dose of levodopa. It is not considered an early event in the course of the dopaminergic treatment and is counteracted by alterations in the dosage or timing of levodopa administration or by the addition of a longer-acting dopamine agonist. The WO is linked to the continuing degeneration of nigrostriatal dopaminergic neurons in the progression of PD. This process is deemed to lead to reduced presynaptic handling and storage of dopamine derived from levodopa, and to a reduced buffering of striatal dopamine receptors from the fluctuations in plasma concentrations of levodopa that occur over the course of the day [36].

Previous reports have demonstrated that approximately 40% of PD patients who were treated with levodopa for 4-6 years experienced WO [37; 38]. The occurrence of WO increases gradually, with almost all PD patients developing WO within 10 years after the initiation of levodopa therapy [38]. Since the occurrence of WO is associated with a decreased quality of life [38], it is important to identify the occurrence of WO and to start managing it immediately. A young age at the time of symptomatic disease onset, high scores on the parts II and III of the Unified Parkinson's disease rating scale (UPDRS) and female sex are reported as the main risk factors related to WO [39].

2.1.2 Levodopa induced dyskinesias (LIDs)

Levodopa is still the main treatment for PD patients. Unfortunately, after a few years of levodopa therapy PD patients develop daily fluctuations in mobility and troublesome, involuntary movements known as levodopa induced dyskinesias (LIDs) [40].

LIDs can be classified into peak-dose dyskinesia (involuntary movements that coincide with the peak-action of levodopa), diphasic dyskinesia (involuntary movements that emerge just before the dopaminergic replacement therapy turns the patient "on" and that reappear at the end of the therapeutic benefit) and "off" period dystonia. The movement disorders most commonly associated with peak-dose LID are chorea, dystonia and ballism. Chorea is characterized by involuntary, irregular, purposeless, non-rhythmic, abrupt and rapid movements that seem to flow from one part of body to the other. Choreic or larger amplitude choreo-athetotic movements are the most common forms of LID. Chorea usually manifests first on the side of the body that is predominantly affected by PD. The severity of choreic movements varies from minor (involuntary movements may not be recognized by patients) to major (involuntary movements may significantly interfere with activities of daily living). Neck and limbs are most commonly affected. Dystonia is the second most common form of LID. It is characterized by sustained contractions of agonist and antagonist muscles that may involve focal/segmental muscle groups. Dystonia as part of LID can be observed as peak-dose dyskinesia, diphasic dystonia or ''off'' dystonia. Diphasic dystonia usually presents as involuntary leg kicking movements. Off-period dystonia is most commonly seen as painful early morning dystonia of one foot or toes. Ballism is characterized by very large amplitude unilateral or bilateral choreic movements of the proximal parts of the limbs. Ballistic movements are usually part of severe choreoathetosis rather than being isolated [41;42].

A study by Rascol and colleagues [43] showed that the incidence of LID in patients with early PD was 45%. The risk factors associated with the development of dyskinesias are gender, age at the onset of disease, rate of disease progression, and dose and duration of levodopa treatment [44].

Numerous important advances have been made in understanding of the etiopathogenesis, pathology and clinical phenomenology of PD and LID over the past 10 years.

However, till today, the neural mechanisms that underlie LID in PD are not completely understood.

The current view on the risk factors underlying the development of dyskinesias suggests that the progression of the dopaminergic degeneration, rather than the duration of levodopa treatment, makes the therapeutic effect to deteriorate over-time [45].

It is known that at the time of diagnosis something between 50 and 70% of nigral dopaminergic neurons have already degenerated; the remaining neurons, however, have the ability to take up the exogenous levodopa, convert it to dopamine (DA), store DA into vesicles, and mediate its synaptic release.

Synaptic levels of DA can be maintained within a physiological range by the presence of D2 auto-receptors and DA transporter (DAT) on striatal DA terminals. The presence of the DA D2 auto-receptor at the pre-synaptic membrane, which activates a feedback control mechanism able to fine-

tune the neurotransmitter release, allows the maintenance of physiological-like synaptic DA levels. Thus, preservation of this mechanism of regulation of DA release in spared DA terminals avoids excessive post-synaptic DA receptor stimulation following chronic levodopa administration in PD patients [46]. Dyskinesias appear to be associated to the inability to maintain synaptic DA levels within certain limits, which is likely caused by the progression of DA neuron degeneration and consequent reduced ability to mediate controlled DA release. The ability of the pre-synaptic DA compartment to prevent excessive DA receptor stimulation, even in presence of supersensitive striatal DA receptors, is also confirmed in rat transplantation studies. In fact, levodopa-primed dyskinetic rats tend to normalize their response to levodopa after receiving ventral mesencephalic DA grafts into the lesioned striatum, which reconstitute the pre-synaptic buffering capacity [47].

Although the efficacy of the treatment is partly compromised in advanced stage of disease, levodopa still produces clear motor effects, of which dyskinesias represent an abnormal manifestation; this suggests that other cellular compartments can substitute the lost DA neurons in mediating levodopa conversion to DA, and neurotransmitter release. In this context, the serotonergic system has emerged, in recent years, as a key player.

Whereas dopamine levels and dopaminergic innervation of the striatum are severely decreased by the time of symptom onset in PD, striatal serotonergic terminal density is only moderately reduced early in the disease and degenerates at a slower pace [48].

Several studies suggest that the compensatory mechanism of the serotonergic system in PD is related to the development of dyskinesia associated with the levodopa treatment [46; 49].

Experimental studies have demonstrated that striatal serotonergic neurons are able to take up, convert exogenous levodopa into dopamine, and subsequently release it from the serotonergic terminals [50-52]. In fact, serotonin neurons share with the dopaminergic neurons, the same enzymatic machinery required to convert levodopa to DA and mediate vesicular storage, the aromatic amino acid decarboxylase, and monoamine vesicular transporter, respectively. However, serotonin neurons lack a feedback control mechanism able to fine-tune the synaptic levels of DA. As consequence, levodopa-derived DA is released in an uncontrolled manner, leading to excessive synaptic DA peaks, and contributing to swings in synaptic DA levels following oral administration of levodopa; this will determine pulsatile stimulation of striatal post-synaptic DA receptors, and changes in signaling cascades at striatal neuron [53; 54]. In particular, uncontrolled stimulation of supersensitized dopamine D1 receptors in the direct striatonigral pathway are thought to mediate LIDs. This theory was first tested in animal models of PD and has been shown that abnormal involuntary movements (AIMs) were alleviated by removing striatal serotonin afferents or by blocking serotonergic transmission with 5-HT1A and 5-HT1B agonists [55-57].

Serotonin release is regulated by somatodendritic 5-HT1A receptors and nerve terminal 5-HT1B receptors. In animal models of PD, 5-HT1A and 5-HT1B receptor agonists act synergistically and can completely eliminate LIDs [54].

Experimental studies have demonstrated that the presence of levodopa-induced AIMs in rats with 6-hydroxydopamine lesions of the nigrostriatal system is critically dependent on the integrity of serotonergic projections. Removing striatal serotonin (5-HT) afferents, or dampening serotonergic activity with 5-HT1A receptor agonists (including buspirone) or 5-HT1B receptor agonists, attenuated AIMs without increasing parkinsonism [57; 58].

A recent PET study has translated into humans the experimental observations. By using 11C-DASB PET to evaluate serotonin terminal function and a series of 11C-raclopride PET assessments to evaluate striatal dopamine release, Politis and colleagues investigated the role of serotonergic mechanisms in the development of LIDs in PD. PD patients with LIDs showed relative preservation of serotonergic terminals compared with those who had a stable response to levodopa. However, identical levodopa doses induced markedly higher striatal synaptic dopamine concentrations in PD patients with LIDs compared with PD patients with stable responses to levodopa. When administration of serotonin receptor type 1A agonist buspirone preceded the administration of levodopa, it reduced the levodopa-evoked striatal synaptic dopamine increases and attenuated LIDs. Importantly, the PD patients with LIDs who exhibited greater decreases in synaptic dopamine after buspirone pre-treatment had higher levels of serotonergic terminal functional integrity [49]. This study provide the first human evidence that striatal serotonergic terminals contribute to LIDs pathophysiology via aberrant processing of exogenous levodopa and release of dopamine as false neurotransmitter in the denervated striatum of PD patients with LIDs.

A large clinical study was conducted in the past years to investigate the efficacy of sarizotan, a partial 5-HT1A receptor agonist, in dyskinetic patients. Despite the promising results obtained in pre-clinical experiments, and in an open-label study, the double-blind investigation was terminated for lack of efficacy [59; 60]. These results may be due to the fact that sarizotan also exerts antagonistic activity at the level of the D2 receptor [61]. Moreover, it should be noted that sarizotan acts only on the 5-HT1A receptor, while experimental evidence demonstrated that a potent synergistic effect on suppression of LID is obtained by simultaneous targeting of the 5-HT1A and 5-HT1B auto-receptors [55].

Of particular interest are recent animal data using eltoprazine, a selective partial agonist at the 5-HT1A and 5-HT1B receptors. Acute administration of eltoprazine reduced LIDs at a low dose (0.3 mg/kg) in 6-hydroxydopamine (6-OHDA) lesioned rats treated with levodopa. In chronic studies, eltoprazine provided protection against the development of LIDs and suppressed already developed

LIDs at doses of 0.3 mg/kg and 0.6 mg/kg. In a non-human primate model of LIDs (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine monkeys treated with levodopa), an acute dose of eltoprazine at 0.75 mg/kg showed suppression of dyskinesias [62].

A recent double-blind, randomized, placebo-controlled and dose-finding phase I/IIa study was conducted by Svenningsson and colleagues [63]. Single oral treatment with placebo or eltoprazine, at 2.5, 5 and 7.5 mg, was tested in combination with a suprathreshold dose of levodopa in 22 PD patients with LIDs. A single dose, oral treatment with eltoprazine has beneficial antidyskinetic effects without altering normal motor responses to levodopa. The most frequent adverse effects after eltoprazine were nausea and dizziness. However, despite these encouraging preliminary results, further clinical studies with chronic oral eltoprazine are needed.

Serotonin 5-HT1 receptor agonists hold promise for the treatment of dyskinesia in PD patients. However, pharmacological silencing of serotonin neurons raises concern for possible negative effects on mood. Indeed, it is well known that most of the advanced PD patients receive treatment with serotonin reuptake inhibitors (SSRIs) for alleviating symptoms of depression [64]. Treatment with selective serotonin autoreceptor agonists would not only reduce the serotonin-dependent DA release, but it is also expected to dampen the synaptic release of serotonin. Moreover, levodopa administration has been shown to reduce serotonin tissue content in rodent models of PD, an effect conceivably due to the competition between DA and serotonin for storage into the serotonin vesicles [54].

A possible strategy to counteract the levodopa-induced reduction of extracellular serotonin levels may be represented by the administration of the serotonin precursor 5-hydroxy-tryptophan (5-HTP). 5-HTP may exert antidyskinetic effect by two possible mechanisms. First, by increasing cytoplasmic serotonin synthesis, which may compete with levodopa-derived DA for storage into the serotonin synaptic vesicles, therefore, reducing serotonin dependent DA release; second, leading to increased activation of serotonin autoreceptors, thus, mimicking the effect of selective serotonin 5-HT1 receptor agonists. Tronci and collagues [65] conducted an extensive characterization of the antidyskinetic effect of 5-HTP in the rat model of LID, showing that acute and chronic administration of the serotonin precursor 5-HTP to levodopa treated 6-OHDA-lesioned rats can significantly reduce established dyskinesia. Importantly, the antidyskinetic effect of 5-HTP does not appear to be due to a competition with levodopa for crossing the blood-brain barrier; in fact, similar levodopa levels were found in the levodopa only and levodopa plus 5-HTP treated groups. Accordingly, it has been reported that 5-HTP easily crosses the blood-brain barrier, and that, opposite to L-tryptophan and other aminoacids, does not require the presence of a specific

transporter. This is also supported by the evidence that 5-HTP did not reduce the therapeutic efficacy of levodopa, as suggested by the results of the motor activity and stepping tests.

2.2 Non-motor symptoms

2.2.1 Rapid eye movement sleep behavior disorder (RBD)

Long before the onset of motor symptoms in PD, a constellation of seemingly unrelated symptoms may arise, including difficulty with smell (hyposmia) and constipation. These symptoms are often subtle and not disclosed to health care professionals.

Patients will describe these phenomena as having been present for decades but of little significance. A more dramatic manifestation of neurodegeneration will arise in the form of violent, often injurious, sleep behaviors. This condition is rapid eye movement (REM) sleep behavior disorder (RBD). RBD is categorised as a parasomnia, the group of sleep disturbances that is characterised by the occurrence of atypical motor, behavioural or cognitive events, occurring at any time between transitioning to sleep and waking. In RBD, patients, typically men aged 60-70 years, do not experience the electromyographic (EMG) atonia that accompanies physiological REM sleep [66].

The pathological lack of atonia is termed REM sleep without atonia (RSWA) [67]. The lack of atonia is associated with complex motor enactment of vivid, often frightening dreams. RBD movements appear non-stereotypical, ranging from non-violent elaborate actions to purposeful aggressive acts because the individual is acting out an internal dream plot; when awoken, patients frequently describe vivid dreams. The spectrum of dream enactment behavior (DEB) ranges from small hand movements to violent actions, such as punching, kicking, or leaping out of bed. Falling out of bed is a common cause of sleep-related injury, often resulting in contusions, with the potential for more serious events that are frequently related to injury of both patients and bed partners, including traumatic brain injuries and subdural haematomas. Bed partners generally describe motor activities during RBD episodes as being more vigorous and normal than when the patient is awake.

Several pivotal studies have shown that patients with so-called idiopathic RBD (iRBD) have an increased risk of developing neurodegenerative disease such as parkinsonism or dementia with risk estimates of 20% to 45% after 5 years [68; 69].

Generally, 50-75% of patients who present with iRBD will go on to develop PD within 10 years of iRBD diagnosis. In 2014, Iranzo and colleagues reported that 90.9% of 174 patients diagnosed with

iRBD, developed a defined neurodegenerative syndrome within 14 years. This powerful finding holds promise for diagnosing PD earlier in the disease course [70-72].

RBD is associated with PD and has found in up to 72% of patients with PD [73]. Despite these high percentages, only a few systematic, large-scale studies have investigated the characteristics of RBD in PD. Clinical features of patients with PD and RBD, and the relationship between RBD and motor symptoms and other non-motor symptoms in patients with PD, are not well described.

There is substantial evidence that PD associated with RBD (PD-RBD) is associated with specific clinical phenotypes, reflecting more severe and diffuse Lewy body disease: older age; longer disease duration; male gender; higher levodopa dose; longer duration of levodopa treatment; greater tendency toward an akinetic-rigid state with lesser tremor predominance; more severe motor phenotype; autonomic impairment; hallucinations and dementia [74-76].

The pathophysiological mechanisms of RBD may be related to dysfunctions of pontine tegmentum, locus coeruleus/sub-locus coeruleus complex and related projections. Studies characterising the location of RBD deficits point to locus coeruleus (LC) and related areas as lesion sites. Since RBD appears well in advance of symptomatic dopaminergic neurodegeneration of SNpc, it is possible that this relationship describes the caudal-to-rostral directionality of a larger disease process.

Regulation of REM sleep involves several key pontine nuclei, especially the glutamatergic subcoeruleus/sublateral dorsal nucleus and REM atonia control is also influenced significantly by the medullary magnocellular reticular formation. The hypothalamus, thalamus, substantia nigra, basal forebrain, and frontal cortex also modulate REM sleep regulation [77].

Physiologic REM twitching follows a predictable temporal pattern, increasing throughout REM sleep periods, mediated by GABAergic and glycinergic inhibitory drive onto motoneurons that steadily declines as REM progresses, enabling more frequent twitching, and suggesting that failure of these GABAergic and glycinergic networks may facilitate pathophysiologic twitching in RBD [78].

According to Braak's theory, this degeneration occurs in the second stage (Braak's stage 2: sublaterodorsal nucleus, magnocellular reticular formation, peri-locus coeruleus) and emerges before the propagation of similar degeneration affecting the dopaminergic-producing neurons in the substantia nigra of the midbrain [26].

A complex interplay of noradrenergic, serotonergic, cholinergic and other neurochemical systems are involved in RBD pathogenesis.

Acute RBD can be induced by the use of antidepressants, especially serotonin reuptake inhibitors (SSRI) suggesting a role of the serotonergic system in the pathogenesis of RBD [79;80].

The cholinergic neurons in the pons are under the inhibitory control of brainstem serotonergic/noradrenergic neurons and they trigger REM sleep by activating the glutamatergic sublaterodorsal nucleus. Then, the glutamatergic pathway activates glycinergic and GABAergic neurons, inhibiting motoneurons as well as brainstem serotonergic and noradrenergic neurons. Thus, the physiological reduction in serotonin release during REM sleep reinforces REM atonia by reducing motoneuron activation, while an abnormal increase in serotonergic tone (possibly due to SSRI) might induce RSWA [81;82].

Serotonergic antidepressants could thus influence motor tone during REM sleep indirectly at brainstem levels (pedunculopontine nucleus or pontine inhibitory area), or directly at spinal levels, producing REM sleep without atonia.

Trazodone (a 5HT1A receptor agonist) has been demonstrate obtain satisfactory results on RBD [83]. Tricyclic antidepressants such as clomipramine, desipramine, amitriptyline and protriptyline, almost all SSRIs (e.g., fluoxetine, paroxetine and sertraline) except for escitalopram, SNRIs such as venlafaxine, duloxetine and zimelidineand, and NARIs such as reboxetine, suppressed REM sleep but elongated sleep latency, increased awakenings, and decreased total sleep time and sleep continuity at the same time [84; 85].

Sleep problems are very common in patients with PD with anestimated prevalence of about 75%, representing a major problem for their quality of life [86].

The serotonergic system is involved in the regulation of sleep and its dysfunction could have relevance to the development of sleep problems in PD.

Preclinical 11C-DASB PET imaging has indicated a direct involvement of serotonergic function in sleep disturbances. Hipólide and colleagues, found a significant decrease in 11C-DASB binding in the anterior olfactory nucleus (22%) and substantia nigra (18%) in rats that have been deprived of sleep when compared to control rats without sleep deprivation [87].

Preclinical studies suggest the brainstem raphe nuclei contribute to an ascending arousal system that promotes wakefulness and prevents excessive daytime sleepiness [88].

A recent imaging study has suggested that cholinergic denervation in cortical, thalamic and limbic structures, rather than dopaminergic or serotonergic denervation, is associated with the occurrence of RBD [11]. In line with these findings, Qamhawi and colleagues did not find any association between raphe serotonin transporter availability and RBD [89].

However, it is important to recognize that normal raphe serotonin transporter binding, seen in the majority of studied patients, does not imply normal serotonergic function in these patients. In fact, the degenerative process in the raphe-striatal neurons in PD could target the nerve terminals rather than the cell bodies.

Published in March 2014, the most recent iteration of the International Classification of Sleep Disorders (ICSD) lists 10 core categories of parasomnias, in which only RBD requires a polysomnography (PSG) for diagnosis [67].

PSG is a commonly performed, multimetric, diagnostic test used to identify sleep-associated illnesses such as RBD: in the setting of a clinical sleep laboratory, physiological information, including EEG, electrooculogram (EOG), electromyogram (EMG) and pulse oximetry, are collected over the course of a night's sleep. The RSWA is the neurophysiologic substrate of RBD, and is required for RBD diagnosis, except when REM sleep is not obtained during a polysomnography recording or is not readily available or is overly expensive.

Visually identified RSWA is of 3 types: phasic/transient (short bursts of muscle activity), tonic (sustained graded increased-voltage background muscle activity), or "any" (either phasic or tonic).

RSWA may be present as an isolated or incidental finding during polysomnography. One recent study found that isolated RSWA without RBD was present in up to 25% of a general community population sample [90].

A single-question screen for RBD, the REM Sleep Behaviour Disorder Single-Question Screen (RBD1Q), has demonstrated impressive sensitivity (93.8%, with 95% CI 90.0 to 96.2) and specificity (87.2%, with 95% CI 82.4 to 90.8), when compared to gold-standard PSG diagnosis, but the RBD1Q is not part of recognised diagnostic criteria [91].

To date, therapeutic approaches have been limited to symptomatic treatment.

The most investigated substances used to treat RBD are clonazepam and melatonin. Numerous larger series and cohort studies have demonstrated good efficacy of clonazepam 0.5–2 mg and is widely accepted among clinicians as a treatment for RBD [92-94]. Melatonin 3-9 mg has also been tested many times openly, as well as in one much smaller study with a double-blind design [95].

Nevertheless, there are patients who do not respond to clonazepam, and the drug can cause side effects such as daytime somnolence, muscle relaxation in the elderly, reduced sleep quality, cognitive impairment and worsening of sleep apnoea.

In a recent open-labeled trial 12 consecutive patients with iRBD were treated with 8 mg ramelteon without clear effect on REM sleep without atonia [96].

Pramipexole is a possible alternative, but limited literature on its effectiveness exists. Overall, the evidence is inconclusive. This is due to the lack of randomised controlled trials [97].

Large multicenter, double-blind, placebo-controlled studies with well-defined outcomes are urgently needed.

2.2.2 Depression

Depression is one of the most common non-motor symptoms in PD and it is estimated to affect approximately 31% of patients [98].

Also, subclinical depression, which can be considered a predictor of major depression (MD), is common among PD patients (54.2%) during the early stages of the disease [99; 100]. Different retrospective studies have shown a positive association between depression and the subsequent emergence of PD [101;102].

Depression can occur throughout the course of PD. The appearance at advanced stages of the disease is possibly a consequence of the widespread neuronal degeneration, a psychological reaction to the physical deterioration and dependence generated by this condition, or both. In contrast, the onset of depression before any clinical sign of PD is not a secondary condition but appears as an integral part of the neurodegenerative process that ultimately trigger the death of dopaminergic (DAergic) neurons. Since the anatomical and neurochemical changes of depression are highly variable among patients, a clear understanding of the pathophysiology of this disease in PD has been elusive. Dysfunctions in the cortico-striato-pallido-thalamic and amygdalo-striato-pallido-thalamic circuits have been associated with the appearance of MD and bipolar disorders. In this regard, lesions of the striatum and orbitofrontal cortex, or degenerative processes of the basal ganglia (BG), both increase the risk for major depressive episodes [103].

The basal ganglia can modulate cognitive and motivational aspects in addition to motor control. The SNpc is not only involved in the motor control of the BG but, as the ventral tegmental area (VTA), is also capable of mediating motivation and reward through the nigrostriatal pathway (SNpc-dorsal striatum) [104]. Dysfunction in this neural network has been associated with depressed mood, anhedonia and apathy [105].

Changes in dopaminergic neurotransmission have been linked to mood disorders in symptomatic PD patients [9]. Several studies demonstrate an association between depressive symptoms and DA loss within the cortical-striatum-thalamocortical circuit at early stages of PD. A retrospective cross-sectional study measuring [123I] FP-CIT binding in 100 PD patients revealed that depressive and motor scores correlate with DA loss in defined regions of the striatum. Depression was also associated with lower DAT binding in the caudate nucleus, while motor symptoms were related to a reduction in DAT binding in the putamen [106]. An additional study showed a negative association between the uptake of [123I] FP-CIT in the striatum and the depression scores [107].

Different findings demonstrate that patients with PD have early alterations of the serotoninergic system and that this could explain the enhanced susceptibility to depression in the disease.

Studies conducted over several decades have shown that in the early phases of PD the level of 5-hydroxyindoleacetic acid (5-HIAA), the primary metabolite of 5-HT, is decreased and is also significantly reduced in PD patients with depression [108].

Changes in 5-HT levels may result from reduced synthesis of the neurotransmitter (caused by dysfunction or death of serotoninergic neurons), increased catabolism, or a combination of both processes. Exogenous levodopa may impair 5-HT function by inhibiting tryptophan hydroxylase and by competing for conversion via aromatic amino acid decarboxylase (AADC) [109; 110].

Moreover, the conversion of levodopa into dopamine by serotonergic neurons has been suggested to result in 5-HT depletion by competing for storage into serotonergic vesicles [111-113]. Hence, chronic levodopa treatment may contribute to the development of depressive symptoms in PD [114].

Imaging studies with Positron Emission Tomography (PET) inferred that the density of 5-HT transporters declines in the striatum of PD patients in a stage-dependent manner [115] whilst a global reduction of presynaptic serotonergic terminals occurs, however without a clear correlation with disease duration, motor disability and chronic exposure to dopamine replacement therapy [29].

In depressed PD patients, cerebrospinal fluid levels of 5-HIAA, the principal metabolite of serotonin, were decreased compared to PD patients without depression [116].

Binding of the radioligand 18F-MPPF to 5HT1A receptors is sensitive to levels of endogenous serotonin. Using 18F-MPPF PET, Ballanger and colleagues have found decreased 5-HT1A receptor availability in limbic regions including the right insula, the left hippocampus, the orbito-frontal region and the uncus in depressed PD patients [117]. These findings are in agreement with post-mortem evidence [118] and support the hypothesis that reductions in postsynaptic 5-HT1A receptors availability could be an underlying mechanism for the development of depression in PD .

Recents PET studies using 11C-DASB, have reported relative increases of SERT binding in limbic structures of depressed PD patients. Boileau and colleagues investigated SERT binding in seven clinically depressed patients with early-stage PD and in seven healthy controls. They found a widespread increase in 11C-DASB binding outside of the striatum. Furthermore, there was a positive correlation between regional increases in 11C-DASB binding and depressive symptoms as assessed by the Hamilton Depression Rating Scale (HDRS). Politis and colleagues also employed 11C-DASB PET in a larger cohort of 34 antidepressant-naive PD patients (10 depressed; 24 non-depressed) and 10 age- and sex-matched healthy controls in order to investigate associations between in vivo serotonergic dysfunction and depressive symptomatology. PD patients with depressive symptoms displayed significantly increased 11C-DASB binding in the amygdala, hypothalamus, caudal raphe nuclei, and posterior cingulate cortex compared to matched-PD patients

without depression but not compared to healthy controls. The 11C-DASB binding increases in these regions in the PD group with depression correlated with depressive symptomatology as measured by Beck Depression Inventory-II (BDI-II) and HDRS [119; 120].

In contrast, in a study involving 45 patients with PD the density of 5-HTT in the midbrain was normal, and there was no correlation with the score of depression measured by the Hamilton Depression Rating Scale [121]. Moreover, this lack of association between 5-HTT availability and the depression score was found in patients with early drug-naive PD [89].

Despite the variability of results, there is an apparent relationship between the increased density of 5-HTT and depression during the early stages of the disease. These findings suggest that reduced extracellular concentrations of serotonin through excessive serotonin clearance by SERT and the concurrent loss of serotonergic terminals in the raphe nuclei and limbic regions could be implicated in the development of PD depression.

Typically used antidepression medication includes tricyclic antidepressants (TCA), selective serotonin reuptake inhibitors (SSRI), serotonin and norepinephrine reuptake inhibitors (SNRI), monoamine-oxidase inhibitors (MAOI), and dopamine agonists (DA). Many clinical trials have been conducted to investigate their therapeutic effect on depression of PD. Barone et al reported in their randomized trial that treatment with rasagiline, an MAOI, did not help to improve depressive symptoms in PD patients [122]. Atomoxetine, an SNRI, was reported to be not efficacious for depression of PD, but might help to improve cognitive disorder and daytime sleepiness [123].

There is a clear consensus that an optimization of dopamine replacement therapy may be the key not only to ameliorate motor disturbances, but also to the successful treatment of depressive symptoms. This conclusion is based on the fact that the only well powered clinical trial addressing depressive symptoms in patients with PD showed that the dopamine agonist pramipexole is highly effective to treat such symptoms. Barone and colleagues conducted a 12-week randomized, double-blind, placebo-controlled trial comparing pramipexole with placebo in patients with mild-to-moderate PD. Two hundred ninety six patients with stable antiparkinsonian therapy without motor fluctuations and depressive symptoms assessed by the Geriatric Depression Scale (GDS ≥ 5) the UPDRS part 1 (depression item score ≥ 2) were included. The primary endpoint was change in the BDI. BDI scores significantly showed a greater improvement in the pramipexole compared to the placebo group. The authors also showed that the effect on depressive symptoms was not only due to improvement of motor impairment. This study can be considered as good evidence for the antidepressant effects of that drug in patients with PD. Yet pramipexole, a DA was found to be able to improve depressive symptoms in patients with PD, through a direct antidepressant effect [124]. A

randomized clinical trial in the USA also found that nortriptyline, a TCA, was efficacious in the treatment of depressive symptoms, but not paroxetine, an SSRI [125].

In a recent systematic review and meta-analysis has been observed that SSRI had a satisfying efficacy for depression of PD patients. They can also help to improve activities of daily living and motor function of patients, yet the adverse effects were also distinctive. SNRI are the safest medication with high efficacy for depression as well. SNRI and TCA are also good at improving depression scores while DA and MAOI tended to have better performance in other symptoms in PD [126].

2.2.3 Apathy

Apathy is a behavioural syndrome that consists of decreased motivation that manifests as a decrease in goal-directed behaviours, and can be variably characterised by reduced interests or emotions that cannot be attributed to diminished level of consciousness, cognitive impairment, or emotional distress [127].

Isolated apathy, which is not related to cognitive impairment or depression, has been recorded at early and advanced stages of PD [128].

Clinical diagnosis of apathy should therefore be based on the presence of symptoms that cause decreased goal-directed behaviours, along with the absence of prominent depressive symptoms.

Four major subdomains of goal-directed behaviour variably contribute (in isolation or, more frequently, in combination) to apathy: reward deficiency syndrome, depression, executive dysfunction, and autoactivation failure. Results from many studies have shown that executive dysfunction is the most consistent neuropsychological correlate of apathy in neurodegeneration [129; 130].

The dysfunction of executive networks makes it difficult to redirect attention to novel stimuli, manipulate complex external or internal information, or generate plans for the future. These deficits lead to a decrease in cognitive interests and a state of so-called cognitive inertia, which restricts the search for new interests [131].

Moreover, apathy might also result from auto-activation deficits, which are the result of an inability to activate oneself or to spontaneously activate mental processing, without external stimulation [132].

Apathy is a frequent neuropsychiatric disturbance that can precede the onset of the first motor symptoms of PD [133]. Depending on the diagnostic methods used, apathy is diagnosed in 20-36% of early PD patients who have not been treated with drugs [134; 135]. In the early stage of PD,

apathy seems to decrease after introduction of dopaminergic treatment, but its frequency increases again to 40% in patients without dementia and to 60% in patients with dementia after 5-10 years of disease [136-138].

Cluster analyses have identified subgroups of patients with PD with predominant or isolated apathy, representing a distinct behavioural syndrome that is associated with specific clinical and demographic correlates, neuropsychological features, and different disease progression.

Specifically, patients with PD who have apathy are more likely to be men and to be older than patients with PD without apathy. Apathy in PD has also been related to more severe motor impairment, worse executive dysfunction, and a higher risk of developing dementia than PD without apathy [139; 140].

Apathy in patients with PD is multidimensional and caused by dysfunction in different neural systems. Both dopaminergic denervation and cholinergic abnormalities affect the lateral and medial regions of the prefrontal cortex in PD. Degeneration of mesencephalic dopaminergic neurons at Braak stage III of the disease leads to dopamine depletion in the mesocortical and mesolimbic systems, and the spread of synucleinopathy to subcortical and cortical dopamine projection areas further impairs their functionality during Braak stages IV and V [26].

Besides having more executive dysfunction, patients with apathy also show impairment in global cognitive function and in performance on cognitive tasks that are more dependent on the temporal lobes (eg, delayed verbal memory, semantic verbal fluency, and confrontation naming) than do patients without apathy [128].

The multidimensional nature of apathy in PD is also clearly evidenced in studies that have comprehensively assessed the presence of depression, apathy, anhedonia, and cognition in patients without dementia. Anhedonia seems to be common to both apathy and depression, making it useless for differential diagnosis in clinical practice. Conversely, whereas apathy seems specifically related to blunted affect, decreased intellectual curiosity, and executive dysfunction, depression seems to be linked to the presence of sadness, dysphoria, negative emotions, and increased anxiety [141-143].

Of particular interest are neuroimaging reports in PD of a dysfunction of dopaminergic transmission in the mesocorticolimbic pathway that might underlie apathetic behaviour. In this regard, PET studies with the dopamine D2 and D3 receptor antagonist [11C]raclopride have shown several differences in dopaminergic binding and transmission between patients with PD with apathy and those without apathy. Notably, [11C]raclopride binding potential was increased in the orbito-frontal cortex, cingulate cortex, dorsolateral prefrontal cortex, amygdala, and striatum in patients with apathy, implying either a reactive increase in D2 and D3 receptor expression or a reduction in endogenous synaptic dopamine, or both [144].

Reported frequencies of apathy range from 15% to 70% in studies of PD [138; 145; 146]. These differences seem to depend on disease severity and on the diagnostic approach used, depend on the diagnosis was established according to cut-off scores on rating scales, instruments rated by caregivers, or clinical diagnostic criteria.

The development of specific scales for apathy has been one of the most important advances in recognition of this issue, both in clinical practice and in research. A task force commissioned by the International Parkinson and Movement Disorder Society reviewed the clinimetric properties of apathy in PD samples [147].

Apathy scales identified for review include the Apathy Evaluation Scale (AES), the Apathy Scale (AS), the Apathy Inventory (AI), and the Lille Apathy Rating Scale (LARS). In addition, item 4 (motivation/initiative) of the Unified Parkinson's Disease Rating Scale (UPDRS) and item 7 (apathy) of the Neuropsychiatric Inventory (NPI) were included. Only the AS was classified as "recommended" by the task force.

The AS is recommended to screen for and to assess the severity of apathy in PD patients; it has proven to be sensitive to change and may be used in treatment studies [147].

Scales that met criteria to be classified but were not recommended were the Apathy Evaluation Scale [127], the Lille Apathy Rating Scale (LARS) [148] and item 7 in the Neuropsychiatric Inventory [149].

Delineation of the different components that can lead to apathy is crucial to the planning of therapeutic strategies.

Pharmacological interventions with cholinesterase inhibitors, dopaminergic drugs (including methylphenidate), and antidepressants have been investigated to improve apathy in PD.

In a recent double-blind, placebo-controlled study of 31 patients with PD with moderate to severe apathy without dementia and depression, a significant improvement in the LARS was reported after 6 months of treatment with rivastigmine at 9,5 mg/day (adjusted effect size 0,9; p=0.031) (Level B, one Class I study according to evidence based medicine assessment) [150]. In an open-label study, the dopamine D2 and D3 receptor agonist ropinirole proved to be efficacious in eight patients with apathy after STN-DBS [151].

Interestingly, improvement of parkinsonian apathy with the dopaminergic drug methylphenidate has been reported as a single case report (Level U) [152] and in another study, where, in a subgroup of seven patients with advanced-stage PD undergoing STN-DBS, administration of high doses of methylphenidate for 3 months was associated with significant reductions in apathy (*p*=0,03), measured using the LARS. However, this study was not specifically designed to assess apathy

[153]. Methylphenidate is known to enhance mesolimbic dopaminergic stimulation by inhibition of the dopamine transporter.

The use of antidepressants for apathy in PD is controversial. Single cases of postoperative parkinsonian apathy after STN-DBS have been reported to be resistant to antidepressive treatment with SSRIs, combined SNRIs, or amitriptyline, but responsive to dopaminergic treatment [154; 155].

In a recent retrospective database and chart review of 181 non-operated PD patients, after controlling for age, sex, education, and depression, the use of SSRIs, have even been reported to increase apathy [156].

Pathophysiological evidence suggests that parkinsonian apathy is primarily due to a mesolimbic dopaminergic denervation, but the role of the serotonergic alteration has never been examined, despite its well-known involvement in the pathogenesis of depression and anxiety.

Data from the study by Maillet and colleagues support the involvement of the serotonergic system also in the development of apathy addressing the pure model of de novo PD, without the confounding effects of antiparkinsonian treatment [10].

Maillet and colleagues used detailed clinical assessment and positron emission tomography imaging, using both serotonergic 11C-DASB and dopaminergic 11C-PE2I PET presynaptic transporter radioligands to index presynaptic serotonergic and dopaminergic function, respectively, in 15 apathetic (Lille Apathy Rating Scale scores $\geq$ -21) and 15 non-apathetic (-36 $\leq$ Lille Apathy Rating Scale scores $\leq$ -22) untreated patients with PD, and in controls. Their findings demonstrate greater serotonergic loss in the basal ganglia in apathetic patients with PD compared to patients without apathy, while both PD groups showed reduced dopaminergic uptake compared to controls. Moreover, greater serotonergic loss in caudate and orbitofrontal cortex correlated with the severity of apathy in patients with PD, whereas apathy was not associated with greater dopaminergic deficits.

Altogether, these findings highlight a prominent role of the serotonergic degeneration in the expression of the neuropsychiatric symptoms occurring at the onset of PD.

The findings of Maillet add apathy to the list of PD motor and non-motor symptoms and complications that are underpinned by serotonergic pathology. Even in this early disease cohort, there was a more pronounced reduction in serotonin transporter (SERT) binding than in dopamine transporter (DAT) binding in those with apathy, thus challenging the concept of predominantly dopaminergic involvement in early disease stages. Maillet and colleagues studied patients with de novo PD. This avoided difficulties in interpreting results in patients who had previously been treated with dopaminergic or serotonergic medications. However, the patients did have elevated

depression and anxiety scores and PET data correlated with both apathy severity and with depression and anxiety levels. Previous PET studies have shown that serotonergic mechanisms play a role in these neuropsychiatric disorders. Disentangling these disorders and identifying the pathophysiology of each remains a challenge. Nevertheless, the results of this study suggest that medications that act on serotonergic targets may be efficacious in the treatment of apathy and other neuropsychiatric disorders in PD.

Chapter 3: 5-HTP

3.1 Pharmacokinetic considerations

5-HTP is an aromatic aminoacid naturally produced by the body from the essential aminoacid L-tryptophan (LT). Produced commercially by extraction from the seeds of the African plant Griffonia simplicifolia, 5-HTP has been used clinically for over 30 years.

The half-life of 5-HTP is relatively short (4.3 ± 2.8 hours) and its time to maximal concentration is 1-2 hours [157-159].

5-HTP is the intermediate metabolite of LT in the production of serotonin (see Fig. 1a). 5-HTP is converted to serotonin by the enzyme aromatic l-amino acid decarboxylase (AADC), an enzyme that catalyzes the decarboxylation of a variety of aromatic l-amino acids; it converts l-3,4-dihydroxyphenylalanine to dopamine and 5-HTP to serotonin [160]. This enzyme acts both in the periphery and in the central nervous system (CNS), meaning that ingested 5-HTP can be converted into serotonin in the periphery of the body. Peripheral serotonin cannot influence CNS serotonin levels because serotonin is unable to cross the blood-brain barrier. Administration of decarboxylase inhibitors such as carbidopa blocks this peripheral conversion. The rationale of carbidopa co-administration is that it prevents peripheral decarboxylation of 5-HTP, leading to more 5-HTP being available in the CNS, and less peripheral serotonin being formed to cause systemic side effects.

While other cells outside the brain, such as blood platelets and some enterocytes, make and/or use serotonin, all serotonin used by brain cells must be made within the neurons, since serotonin cannot cross the blood-brain barrier. Therefore, the synthesis of serotonin is heavily dependent upon the availability of LT within the CNS.

Therapeutic use of 5-HTP bypasses the conversion of LT into 5-HTP by the enzyme tryptophan hydroxylase, which is the rate-limiting step in the synthesis of serotonin. Tryptophan hydroxylase can be inhibited by numerous factors, including stress, insulin resistance, vitamin B6 deficiency, and insufficient magnesium. In addition, these same factors can increase the conversion of LT to kynurenine via tryptophan 2,3-dioxygenase, making LT unavailable for serotonin production.

5-HTP is well absorbed from an oral dose, with about 70% ending up in the bloodstream [161;162]. Serotonin levels in the brain are highly dependent on levels of 5-HTP and LT in the CNS. 5-HTP easily crosses the blood-brain barrier, not requiring the presence of a transport molecule. Absorption of 5-HTP is not affected by the presence of other aminoacids; therefore, it may be taken with meals without reducing its effectiveness. LT, on the other hand, requires use of a transport

molecule to gain access to the CNS. Since LT shares this transport molecule with several other aminoacids, the presence of these competing aminoacids can inhibit LT transport into the brain.
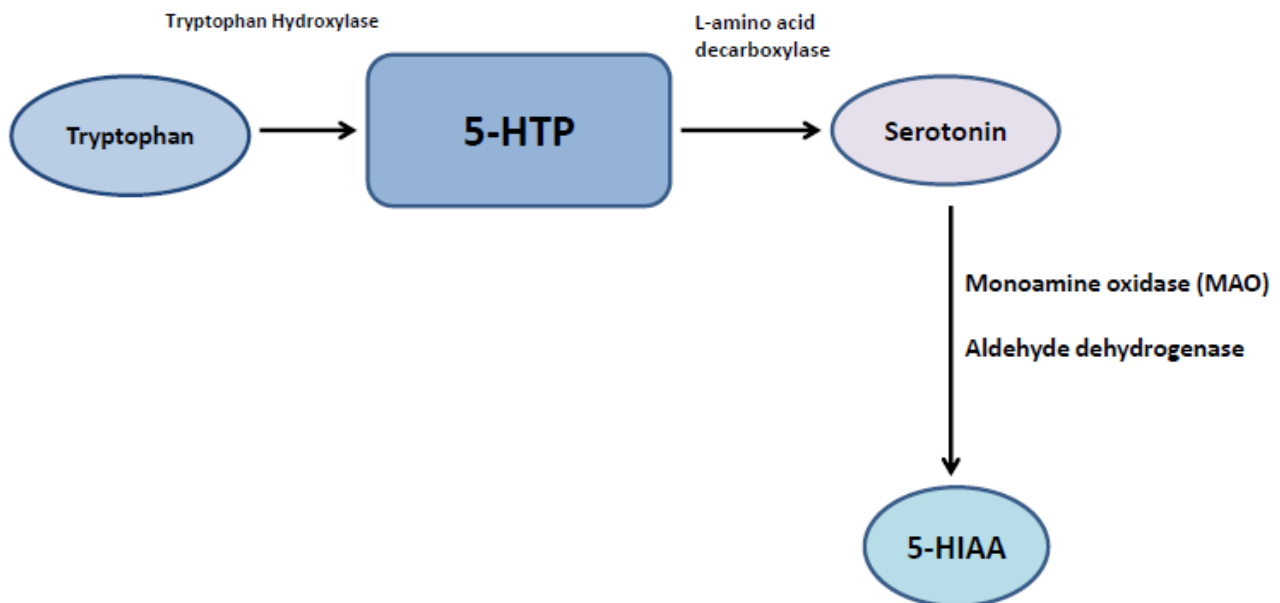

Fig. 1a. Flow chart illustrating the biosynthesis and metabolism of 5-HTP. The enzymes involved in the process are shown to the right of the arrows. Abbreviations: 5-HTP: l-5-hydroxytryptophan; 5-HIAA: 5-hydroxyindoleacetic acid.

3.2 Efficacy of 5-HTP: clinical evidences

Much of the published research on 5-HTP has to do with its use in the treatment of depression. Definitive, large-scale studies of efficacy and safety have not been conducted for 5-HTP. A total of 27 studies have evaluated the efficacy of 5-HTP for depression (total N=990). These studies were heterogeneous in terms of design, duration and dose. Eleven of these studies were double-blind, placebo-controlled and the 5-HTP was superior to placebo in 7 of them [163-173]. However, the sample sizes in all these studies were quite small, and only 5 of these studies were able to show statistical significance. Of these, one was a monotherapy relapse prevention study [170], three were augmentation studies [163; 166; 172], and one tested a 5-HTP/dopamine agonist combination against placebo, rather than 5-HTP alone [167]. Only a few studies, most of them augmentation studies, were of sufficient quality to show statistical superiority to placebo.

The current conventional therapies of choice for depression are the SSRIs. Poldinger W. and colleagues evaluated 5-HTP in comparison to an SSRI drug in a double-blind, multicenter study design. A total of 36 subjects, all of whom were diagnosed with some form of depression, received either 100 mg of 5-HTP three times per day, or 150 mg of fluvoxamine (an SSRI) three times daily. The subjects were evaluated at 0, 2, 4, and 6 weeks, using four evaluation tools: the Hamilton Rating Scale for Depression (HRSD), a standard depression rating scale; a patient-performed self-assessment; the investigator's assessment of severity; and a global clinical impression. Both treatment groups showed significant and nearly equal reductions in depression beginning at week two and continuing through week six. After four weeks, 15 of the 36 patients treated with 5-HTP, and 18 of the 33 patients treated with fluvoxamine had improved by at least 50 percent, according to the HRSD scores. By week six, the two groups had about equal numbers showing 50 percent improvement. When the numbers were totaled at the end of the study, the researchers found the mean percentage improvement from baseline to the final assessment was slightly greater for patients treated with 5-HTP. The number of treatment failures was higher in the fluvoxamine group (5/29, 17%) than in the 5-HTP group (2/34, 6%), although neither of these differences were statistically significant. All four evaluation tools yielded similar results.

Overall, 5-HTP appeared to be slightly better tolerated than fluvoxamine, although the results did not reach the level of statistical significance [174].

Primary fibromyalgia syndrome is characterized by general musculoskeletal aching, multiple tender points, fatigue, morning stiffness, and sleep disturbances. Fibromyalgia patients have been found to have low serotonin [175]. These findings suggest 5-HTP might be useful in the treatment of fibromyalgia, and three clinical trials have demonstrated significant improvement in symptoms, including pain, morning stiffness, anxiety, and fatigue. In a longer-term study, a total of 50 patients diagnosed with primary fibromyalgia syndrome were given 100 mg 5-HTP three times per day for 90 days in an open study. Patients were assessed at the beginning of the study and after 15, 30, 60, and 90 days of treatment. The clinical variables evaluated included: total number of tender points, pain intensity, sleep quality, morning stiffness, anxiety, and fatigue. All of these measures showed significant improvement throughout the length of the study ($p<0.001$). A total of 15 patients (30%) reported side effects from the 5-HTP, but in only one case were they severe enough for the patient to be withdrawn from the study [176].

During dieting, serum tryptophan levels and CNS serotonin levels drop dramatically [177]. These low serotonin levels in obese patients have been associated with carbohydrate cravings and resultant binge eating. It has been theorized that 5-HTP can help prevent this dieting-associated decline in

serotonin, thus enhancing weight loss. Three clinical trials in obese patients have demonstrated decreased food intake and subsequent weight loss with 5-HTP supplementation [178;179].

Chronic headaches, especially migraines, are considered by some researchers to be the result of low serotonin levels, probably as the result of increased breakdown of serotonin by the enzyme monoamine oxidase [180]. 5-HTP has been used successfully in the prevention of chronic headaches of various types, including migraine, tension headaches, and juvenile headaches [181;182].

The 5-HTP has been shown to be beneficial in treating insomnia, especially in improving sleep quality by increasing REM sleep. Eight normal subjects were monitored to determine the effect of 5-HTP on REM sleep. A total of 600 mg 5-HTP was administered to the subjects in the following manner: 200 mg at 9:15 pm, followed by 400 mg at 11:15 pm. A significant increase in the amount of REM sleep was observed while the subjects were taking 5-HTP (118 ± 14 mins vs. 98 ± 11 mins, $p<0.005$). A smaller study using a 200 mg dose also showed increases in REM sleep, but to a lesser degree [183-185].

Review of the literature reveals a lack of data on dosage and usage considerations. The doses used in the studies ranged from 20 to 3250 mg/day, with the majority administering 5-HTP at doses between 200 and 300 mg/day, regardless of whether carbidopa or another medication was co-administered.

The most common adverse effects of 5-HTP are gastrointestinal and include nausea, vomiting, and diarrhea. Less commonly, headache, insomnia, and palpitations can occur. A study comparing 5-HTP to 5-HTP plus a peripheral decarboxylase inhibitor found that gastrointestinal side effects were dose dependent and that they occurred more frequently in patients receiving 5-HTP alone. This may be due to peripheral conversion of 5-HTP to serotonin, which increases gut motility. Such conversion is blocked by peripheral decarboxylase inhibitors.

Patients with PD receive treatment with levodopa-carbidopa, a combination of the levodopa and decarboxylase inhibitor carbidopa. By blocking peripheral conversion of levodopa to dopamine in the periphery, more levodopa penetrates the blood-brain barrier. The same rationale exists for administering carbidopa along with 5-HTP. In a study of healthy volunteers, the addition of carbidopa resulted in a 14-fold increase in 5- HTP plasma levels [186].

Serotonine syndrome is a theoretical possibility with any drug that affects the serotonin system, including SSRIs and tricyclic antidepressants. To our knowledge, however, serotonin syndrome has not been reported in humans in association with 5-HTP, either as monotherapy or in combination with other medications. Regarding the combination of 5-HTP with SSRIs, we know of only 1 relevant study [187].

Chapter 4 : Materials and Methods

This study has been approved by the Local Ethics Committee. The study has been registered in the Eudra CT database and a written informed consent was obtained from all subjects.

The study protocol was conducted at the Sleep Disorder Centre and Movement Disorders Centre - University of Cagliari, Cagliari, Italy.

4.1 Study design and participants

This is a single-center, randomized, double-blind (subject/investigator), two-period crossover study design.

A total of 66 consecutive patients from the outpatient clinic in Cagliari, diagnosed with idiopathic PD according to the UK Brain Bank Parkinson's Disease criteria [188], were screened for inclusion. Recruitment was performed by specialist movement disorder clinics at the Movement Disorders Centre of the University of Cagliari. Exclusion criteria included vascular parkinsonism (defined by evidence of relevant cerebrovascular disease, as indicated by brain imaging computed tomography (CT) or magnetic resonance imaging (MRI), or by the presence of focal signs or symptoms that are consistent with stroke); brain tumor; drug-induced parkinsonism (neuroleptic treatment at onset of symptoms); other known or suspected causes of parkinsonism (e.g. metabolic, etc.), or any suggestive features of a diagnosis of atypical parkinsonism; severe dementia as defined by a MoCA (Montreal Cognitive Assessment) [189] score ≤ 18; severe speech problems and poor general health; concomitant neurologic and/or psychiatric diseases; depressed patients receiving SSRIs or SNRIs; participation in other drug studies or receipt of other investigational drugs within 30 days prior to baseline; in the investigator's opinion, any unstable or clinically significant condition that would impair the participants' ability to comply with a long term study follow-up; shift workers, who cannot ensure traditional night time sleep habits.

Thirty six patients were subsequently enrolled in the study after giving their written consent. Before randomization, every patient underwent a clinical interview, neurological, neuropsychological, and psychiatric assessment. Between September 16, 2016, and February 14, 2017, eligible patients were randomized on day 1 in a 1:1 ratio to receive placebo or 5-HTP.

Placebo and 5-HTP capsules and packaging were identical in appearance.

As schematically illustrated in Fig. 1b, the study included six study visits for each patient. At the second visit (baseline), the patients were randomized into the study and assigned a specific study

number. At the second visit, the patients initiated their first treatment period, receiving either 5-HTP or placebo (we referred to this part of the study as Part I), according to their randomization, until their third visit. Following a washout period of 4 weeks, the patients crossed over to receive the alternative intervention for 1 month, placebo tablet (matching 5-HTP) or 5-HTP 50 mg tablet, respectively, once daily (we referred to this second part of the study as Part II).

We decided for a washout period of 4 weeks in order to minimize the carry-over effects.

Patients were assessed at screening and baseline (week 0), and at weeks 4, 8, 12 (end of treatment), and 16 (T-end).

Throughout the entire study, patients remained in their natural environment and were asked not to change their daily routines.

Patients were provided with 5-HTP (50 mg per capsule) and placebo in sealed and coded packages, inserted into identically appearing capsules. Patients, investigators, or other study personnel were not aware of a patient's treatment assignment. The treatment was administered once a day during the night, within 30 minutes before bedtime and approximately 45 to 60 minutes after the last daily dose of levodopa. A small group of subjects with LIDs were randomly assigned to receive 5-HTP 50 mg/day or placebo orally once a day in the morning, 30 minutes after the first daily dose of levodopa. No changes in antiparkinson medications and other psychoactive drugs were allowed during the study.

Treatments were identified by a number related to each sequence.

Safety assessments conducted throughout the study period included reports of any adverse events experienced by the patients or reported by parents, and vital signs recorded by the physician.
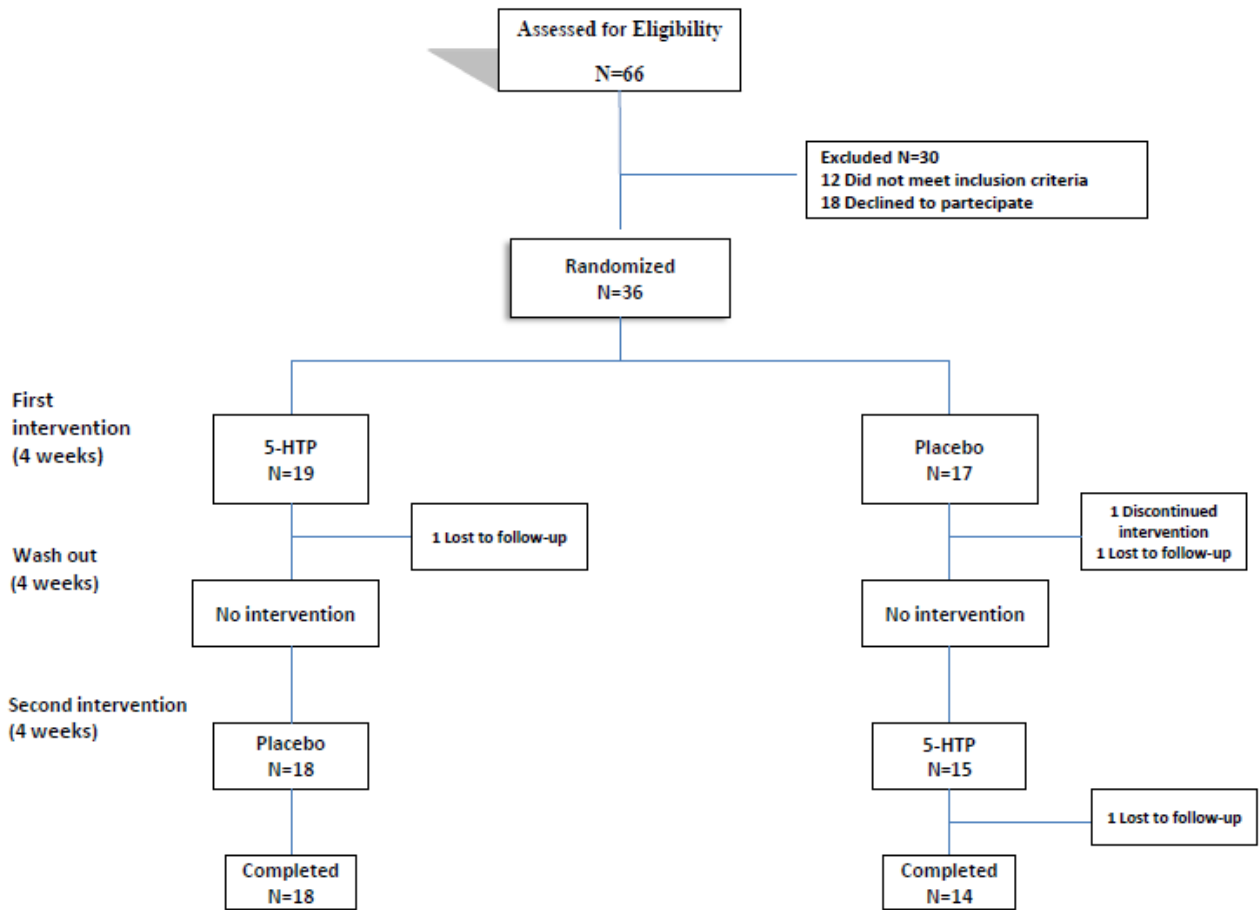
Fig.1b Enrollment, Randomization, and Retention of Study Participants.

4.2 Assessments

At each visit subjects were placed in a quiet room where evaluations were performed. At screening and baseline evaluation, demographic data, disease characteristics and medication (with particular attention to levodopa and dopamine agonist), the presence of comorbidities and past medical history were being collected during a face-to-face interview. The levodopa equivalent daily dose (LEDD) was calculated for each patient [190].

4.2.1 Overall functional status

For the assessment of efficacy on the overall functional status, Unified Parkinson's Disease Rating Scale (UPDRS, Parts I, II, III) scores were obtained at baseline, and weeks 4, 8,12 and 16 (T-end) [191]. UPDRS Part III (motor examination) assessments were made 2-3 h after the patient took a scheduled dose of levodopa. The UPDRS is a 41-item instrument divided into four parts: Part I, mentation, mood and behavior; Part II, activities of daily living; Part III, motor examination and Part IV, motor fluctuations and dyskinesias [191].

4.2.2 RBD assessment

For the assessment of efficacy on the RBD, video-polysomnography (v-PSG) was performed at baseline, and home-based polysomnography was performed at weeks 4 and 12.

Polysomnographic recordings

All patients underwent one full-night attended video-polysomnography (video-PSG) recording in sleep laboratory with digital polysomnography according to the American Academy of Sleep Medicine (AASM) recommendations [192]. Video-PSG was performed with digitally synchronized videography and the following montage was employed: electroencephalographic leads (F3-A2, F4-A1, C3-A2, C4-A1, O1-A2, O2-A1), left and right electrooculography (EOG) channels, bilateral surface EMG channels (submentalis, flexor digitorum superficialis on upper limbs, tibialis anterior on lower limbs), and electrocardiography. The respiratory analysis included nasal airflow, which was recorded by both thermistor and nasal pressure sensor, thoracic and abdominal respiratory effort, oxygen saturation recording by cutaneous finger pulse-oxymeter and microphone. Patients were asked to sleep uncovered in order to improve the detection of motor activity, but a light sheet could be allowed for their comfort.

Sleep stages were scored according to American Academy of Sleep Medicine (AASM) criteria,[192] with allowance to chin EMG muscle tone during REM sleep. The following sleep data were collected for descriptive purpose: total sleep time (TST), sleep efficiency (SE), wake after sleep onset (WASO), percentage of time in each sleep stage (N1, N2, N3, REM), number of REM sleep episode and arousal index.

Diagnosis of RBD

RBD diagnosis was made by fully trained neurologist blinded to clinical patient's status according to the International Classification of Sleep Disorders third edition (ICSD-3),[67] including a quantitative measure of RSWA, namely "any chin EMG activity, tonic and/or phasic, combined with bilateral phasic activity of the flexorum digitorum superficialis (FSD) muscle" in ≥27% of REM sleep scored in 30-s epochs. The rational to choose this cut-off, based on the SINBAR method,[193] as reference standard, relies on the fact that the latter has been included in the ICSD-3 "as the most current evidence-based data for detecting RSWA in the evaluation of RBD, reliably distinguishing RBD patients from controls". Patients were excluded from the analysis if they had spent less than five minutes in REM sleep, since this REM duration was believed to be insufficient for a reliable assessment of RSWA. Each video-recorded REM sleep period was carefully analyzed in order to detect any motor behaviors or sleep vocalizations referable to RBD, such as violent and non-violent motor complex activity. The EMG activity of the chin and bilateral FDS were analyzed. REM sleep epochs were carefully examined for artifacts, and increases in EMG tone caused by respiratory arousal were excluded. The minimum amplitude of EMG activity during NREM sleep was considered as the background EMG activity for each patient. The EMG signal was analyzed with a notch filter at 50 Hz and rectified. Visual scoring was performed by a single sleep-specialist scorer (MF), who was blinded to RBD history. Details of RSWA scoring are described in Figorilli et al. [194].

Severity assessment of RBD

Severity and frequency of RBD episodes were assessed on the basis of a "RBD severity scale" (RSS) filled in by the patient.

The RSS establishes overall severity of disease at a single time point through 6 questions on frequency (0-4 point scale), and severity (1-4 point scale). The RSS covers questions about the frequency and severity of dream acting problems, disturbing dreams or nightmares and how often the patient talks loudly, yells, hits or kickes during the sleep.

It can be used as an index of change for clinical trials and needs a shorter time course, generally one month. RSS is able to be used both by people who are alone and those with bed partners. This scale it is not used to make diagnosis but only to rate severity in those cases in which the diagnosis has already been made.

4.2.3 Depression and Apathy

To measure the depression status, the Beck Depression inventory (BDI-II) and the 21 - item version of Hamilton Depression Rating Scale (HDRS$_{21}$) were used [195;196].
The BDI-II is one of the most widely used screening tool for depression. The BDI-II included 21 questions for measuring the symptoms of depression, including sleep disorders, appetite, self-confidence, hope, and feelings of sadness. Each question had four options ranging from 0 to 3. The score was based on the number of each choice. Eventually, a total score was obtained out of the 21 questions, ranging from 0 to 63. The standardized cut-off commonly used are the followings: 0 to 13, normal; 14 to 19, mild depression; 20 to 29, moderate depression; 30 to 63, severe depression.

A total of 22 patients were assessed with the HDRS$_{21}$. The HDRS$_{21}$ is the most widely used clinician-administered depression assessment scale. The time required to administer the HDRS$_{21}$ is about 20-30 minutes. The original version contains 17 items (HDRS$_{17}$) pertaining to symptoms of depression experienced over the past week. Although the scale was designed for completion after an unstructured clinical interview, there are now semi-structured interview guides available. The HDRS was originally developed for hospital inpatients, thus the emphasis on melancholic and physical symptoms of depression. A later 21-item version (HDRS$_{21}$) included 4 items intended to subtype the depression. A limitation of the HDRS is that atypical symptoms of depression (e.g., hyperphagia, hypersomnia) are not assessed. Although the HDRS form lists 21 items, the scoring is based on the first 17. Eight items are scored on a 5-point scale, ranging from 0 = not present to 4 = severe. Nine are scored from 0-2. Since its development the scale has been widely used in clinical practice and become a standard in pharmaceutical trials. A score of 0-6 is considered to be normal. Scores of 7 or higher indicate mild, moderate, or severe depression.
To measure the apathy status, the Apathy Scale (AS) [145] was used. The AS is an abbreviated version of the Apathy Evaluation Scale [127] developed specifically for patients with PD, and with proven reliability and validity in assessing apathy in patients with PD. The AS has shown to be sensitive to change during pharmacological treatment [147].

The AS consists of 14 questions that are answered by the patient or caregiver (where appropriate) on a 4-point scale. The total AS score is calculated by summing the single scores, with higher scores indicating more severe apathy; a total score of ≥14 is indicative of clinically relevant apathy symptoms.

For the assessment of efficacy on apathy and depression the AS; the BDI-II and the $HDRS_{21}$ were performed at baseline, and weeks 4, 8, 12 and 16 (T-end).

4.2.4 Levodopa-induced dyskinesia (LID) and motor fluctuactions

Levodopa-induced dyskinesia (LID) and motor fluctuations have been assessed through the Unified Dyskinesia Rating Scale Part 1 A/B (UDyRS) [197], UPDRS (Section IV) [191] and the Wearing-Off Questionnaire (WOQ-19) [198] at baseline, and weeks 4, 8,12 and 16 (T-end).

The UDyRS Part 1 assesses the presence and impact of on-dyskinesia on patients' experiences of daily living. There are 11 questions. Part 1A is administered by the rater and there is one question that focuses on the time spent with on-dyskinesia (0 = Normal: No dyskinesia; 1 = Slight: ≤ 25% of on-time; 2 = Mild: 26-50% of on-time; 3 = Moderate: 51-75% of on-time; 4 = Severe: > 75% of on-time). Part 1B is a component of the questionnaire that covers 10 questions on the impact of on-dyskinesia on experiences of daily living (speech; chewing and swallowing; eating tasks; dressing; hygiene; handwriting; doing hobbies and other activities; walking and balance; pubblic and social settings). Off-dystonia is not considered.

The diagnosis of Wearing-Off (WO) was made on both the neurologist evaluation and the basis of the patient self-assessment using the Italian version of the 19-item Wearing-Off Questionnaire (WOQ-19). The WOQ-19 was shown to be a potent screening tool for WO in patients with PD. The WOQ-19 consists of 9 items assessing fluctuations of motor symptoms, including tremor, difficulty in speech, weakness, problems with balance, slowness, reduced dexterity, general stiffness, muscle cramps, and difficulty getting out of the chair and 10 items assessing fluctuations of non-motor symptoms, including anxiety, sweating, mood changes, numbness, panic attacks, cloudy mind/dullness of thinking, abdominal discomfort, experience hot and cold, pain, and aching. For each item, patients were asked to tick whether symptoms were present and whether they improved after the following dose of levodopa treatment: a cut-off of ≥2 improved symptoms had been previously established to make diagnosis of WO [199].

Measures of dyskinesia severity were also obtained from the self-reported 24-hour home dyskinesia diaries. Patients were asked to provide at least 21 days of valid patient diary data during treatment. To ensure reliable and accurate completion of these diaries by each patient, extensive instructions

were given. Patients were instructed to place one check mark on each diary to indicate their predominant clinical status every hour while awake. The diary included the following categories: asleep; severe rigidity; moderate rigidity; mild rigidity; "on" without dyskinesia; "on" with mild dyskinesia; "on" with moderate dyskinesia and "on" with severe dyskinesia. Written instructions and descriptions of each category were included in the diaries.

4.2.5 Safety assessments

Safety assessments conducted throughout the study period included reports of any adverse events experienced by the patients or reported by parents, and vital signs recorded by the physician.

4.2.6 Medication use

Current medication use is reviewed at each assessment. Since dopaminergic agents can have a positive influence on motor (motor fluctuations and dyskinesias) and non-motor (sleep; apathy and depression) symptoms, we controlled for the total dose of dopaminomimetics, which is converted to a levodopa equivalent daily dose.

4.3 Outcome measures

4.3.1 Primary efficacy outcomes

- The effect of 5-HTP on the the percentage of RSWA compared to placebo.
- The effect of 5-HTP on TST; SE; WASO; arousal index and percentage of time in each sleep stage (N1, N2, N3, REM) compared to placebo.
- The comparison of 5-HTP to placebo in mean change from baseline to week 4, 8, 12 and 16 in total score on the AS; BDI-II and $HDRS_{21}$, as assessed by two independent and blinded investigators.
- The comparison of 5-HTP to placebo in mean change from baseline to week 4, 8, 12 and 16 in UDysRS Part 1A/B; UPDRS (Part IV) and the WOQ-19, as assessed by two independent and blinded investigators.

Efficacy assessments were done at baseline, 4, 8, 12 weeks and 1 month after the study treatment (week 16).

4.3.2 Secondary efficacy outcomes

- The comparison of 5-HTP to placebo in mean change from baseline to week 4, 8, 12 and 16 in the total score of a self-administered RSS filled in by the patient and by his/her bed partner.

- The comparison of 5-HTP to placebo in change from baseline to week 4, 8, 12 and final visit on the overall functional status as reported by the UPDRS (Part I, II and III) scores.

- The comparison of 5-HTP to placebo in changes in mean daily "on" hours with mild, moderate and severe dyskinesia and changes in mean daily hours spent in the off -state (with mild, moderate and severe rigidity) while awake, as recorded in the patient diaries. Mild dyskinesia was defined as dyskinesia that did not interfere with function or cause significant discomfort [200].

Chapter 5: Statistical Analysis

Descriptive statistics (means and standard deviations for quantitative variables, or frequencies and percents for categorical variables) were obtained to describe variables at baseline time point.

Most of our variables do not meet the assumptions of parametric statistical tests; indeed, using a parametric statistical test on such data may give misleading results. Accordingly, in order to perform parametric tests, percent transformations (we set the baseline values at 100% and then we transformed the other values considering the percent variation from baseline) were made on all data sets from clinician and patient-administered rating scales.

To evaluate the effect of 5-HTP and placebo on all of the primary and secondary outcome measures across all time points, we performed the Anova's Two-way Analysis of Variance with time as the within-subject factor and treatment (5-HTP or placebo) as the between-subject factor. Additionally, between-group differences in different treatment conditions (5-HTP versus placebo) were analyzed using the unpaired t-test.

Moreover, in case of the absence of time effects across all time points we performed the one - way Anova Analysis for repeated measures in order to evaluate the differences between all 5-HTP (Part 1 and Part 2) and placebo (Part 1 and Part 2) treatment conditions compared to baseline data from both groups.

In each single experimental group (5-HTP-placebo and placebo-5-HTP), scores in the different treatment conditions (5 HTP treatment vs placebo) were evaluated by means of parametric one-way Anova test for repeated measures, followed by the t-test applied to selected paired comparison of mean values.

The patient diary data in the different treatment conditions (5-HTP versus placebo) were analysed using the Anova's Two-way Analysis of Variance with "severity" as the within-subject factor and "treatment" (5-HTP or placebo) as the between-subject factor.

All Post hoc comparisons were performed by 2 tailed Bonferroni test when possible, according to the significance of the main factors or their interaction.

All subjects who completed the protocol were included in the analyses.

Chapter 6: Results

Out of the 66 patients screened for eligibility between November 2015 and March 2016, 54,5% (N=36) were randomized to intervention and 32 completed both crossover periods. Three subjects withdrew from the study after random allocation due to transportation issues. One patient missed the last evaluation at week 16 due to moderate dehydration that required urgent hospitalization.

Seventeen patients had been assigned randomly to placebo-5-HTP arm, and 19 patients had been assigned randomly to 5-HTP-placebo arm.

None of the 33 patients evaluated in our study failed to take their medication more than once during any one treatment period.

A total of thirty-three patients (22 men, 11 women) were included in the final analysis; mean age was 68,1 years with a disease duration of 8.7 years. Their demographic and baseline clinical informations are given in Table 1.

Table 1.

Demographic and clinical informations	
Age (years)	68.18 ± 7.2 (54-83)
Gender (M/W)	22 (66.6%)/ 11(33.3%)
PD duration (years)	8.75 ± 5.75 (2-24)
Levodopa equivalent dose (mg)	621 ± 436.7
UPDRS I	2.18 ± 2.51 (0-13)
UPDRS II	10.12 ± 7.59 (0-32)
UPDRS III	17.42 ± 9.71 (4-46)
UPDRS IV	3.75 ± 4.43 (0-15)
Total UPDRS	33.48 ± 19.68 (7-83)

Data are presented as mean and standard deviation (S.D); Abbreviations: UPDRS, Unified Parkinson's Disease Rating Scale.

All patients were on levodopa and/or dopamine agonists and mean levodopa equivalent daily dose was 621 ± 436.7 mg/day. At baseline evaluations 8 (24.24%) patients were on levodopa monotherapy; 2 (6%) patients were on levodopa in combination with Catechol O-Methyltransferase inhibitors (COMT-i); 1 (3%) patient was on levodopa and MAO-B inhibitor (MAO-i); 12 (36.36%) patients were on levodopa in combination with dopamine agonist (DA); 5 (15.1%) patients were on

levodopa in combination with DA and MAO-i; 3 (9%) patients were on DA monotherapy; 1 (3%) patient was on DA and MAO-i and 1 (3%) patient was on DA in combination with COMT-i.

All subjects were maintained on stable doses of antiparkinsonian medications during the course of the study.

Participants enrolled in the study had mild to severe PD: mean UPDRS Part 1 was 2.1 ± 2.5; mean UPDRS Part 2 was 10.1 ± 7.5; mean UPDRS Part 3 was 17.4 ± 9.7; mean UPDRS Part 4 was 3.7 ± 4.4; mean total UPDRS was 33.4 ± 19.6.

Nine (27.2%) patients were akinetic-rigid subgroups and twenty (60.6%) patients were tremor-dominant and four (12.1%) patients were mixed subgroup. Mean Montreal Cognitive Assessment (MoCA) score = 25±2.92. Mean Frontal Assessment Battery (FAB) scores = 14.4±2.7.

Of the 33 patients who were screened and received treatment, 12 (36.3%) had LIDs (6 men; 6 women) and motor fluctuactions and 7 (21.2%) had motor fluctuactions without LIDs. Out of these 12 patients a total of 5 had RBD as assessed by the v-PSG. On average, the number of years with levodopa treatment was 11.17 (7-16), and with a duration of dyskinesia of 5.08 years (2-9). The total equivalent daily dosage of levodopa was 919 mg (range 248.3-1815), in different combinations with DA, MAO-i and COMT-i.

According to the BDI-II, at the baseline evaluation, mild - moderate and severe depression (BDI-II ≥14) was present in 10 (30.3%) patients (5 men; 5 women); mean age 68.3±7.8; mean disease duration 9.1±4.4; mean total LEDD 696.1±512.1. According to the $HDRS_{21}$, at the baseline evaluation, mild-moderate and severe depression was present in 17 (77.3%) patients (11 men; 6 women); mean age 66,3±6.1; mean disease duration 11.7±5.2; mean total LEDD 773.3±487.0.

At the baseline evaluations, according to the AS, a total of 12 (36.36%) patients presented apathy symptoms (9 men; 3 women); mean age 68.3±6.8; mean disease duration 7.8±5.3; mean total LEDD 442.8±310.5.

At baseline evaluations, according to v-PSG, a total of 17 (51.5%) patients were diagnosed with RBD (11 men; 6 women); mean age 67.9±7.4; mean disease duration 8.2±4.8; mean total LEDD 750.2±516.2. A total of 8 (47%) patients had been assigned randomly to 5-HTP in Part I, and 9 (53%) patients had been assigned randomly to 5-HTP in Part II.

No significant differences in baseline characteristics were detected between the 2 treatment arms.

6.1 Changes in primary outcomes:

6.1.1 Sleep variables

There were no significant main effects neither for the treatments (5-HTP vs placebo) nor for time in all sleep variables evaluated at any of the assessment time points as reported by Two-way analysis of variance (ANOVA).

Compared to baseline, 5-HTP didn't reduce significantly the percentage of RSWA.

Treatment with 5-HTP significantly (p=0.04; t=2.456 df=7) reduced the percentage of time in stage N3 compared to placebo (see Fig.2). The 5-HTP did not change phasic muscle activity during REM sleep, but did increase the percentage of time in REM sleep stage as compared to placebo (see Fig. 3,4). Analyses also revealed that 5-HTP produced a marginal, non-significant reduction in arousal index, WASO and percentage of time in stage N1 vs. placebo (see Fig.5,6,7). There were no other significant effects of 5-HTP at dose 50 mg compared to placebo on nighttime sleep parameters evaluated in our study. Results from the PSG data are presented in Table 2.

Fig.2
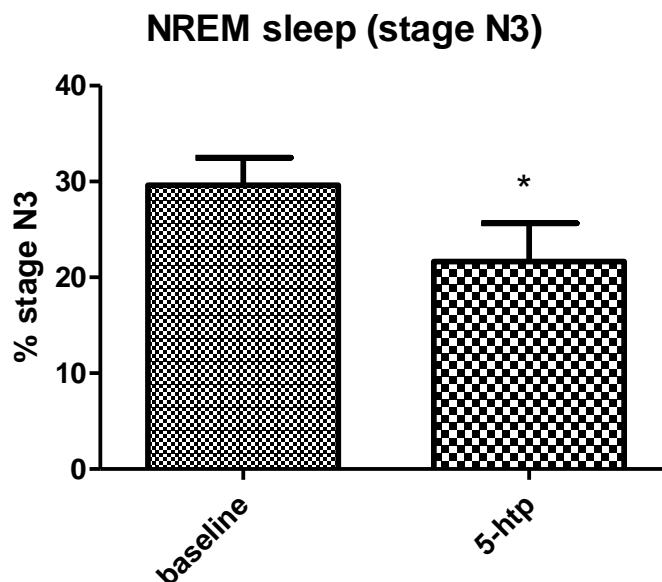
Polysomnographic sleep data: % stage N3 (NREM) (primary outcome). Paired t-test (p=0.04; t=2.456 df=7)

Fig.3
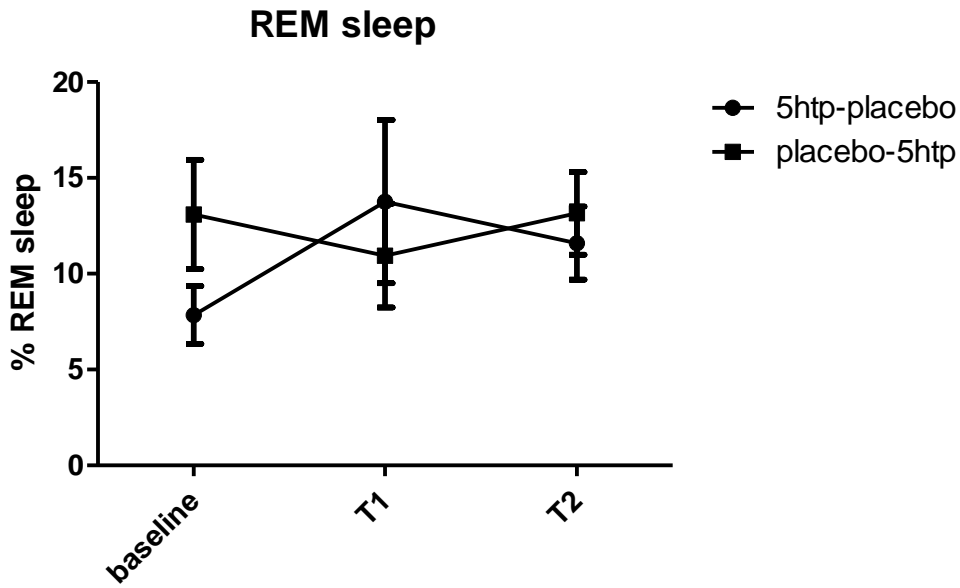
REM sleep

Polysomnographic sleep data : % REM sleep (primary outcome). Two way anova (treatment p=0.6; F=0.2; time p=0.6; F=0.4)

Fig.4
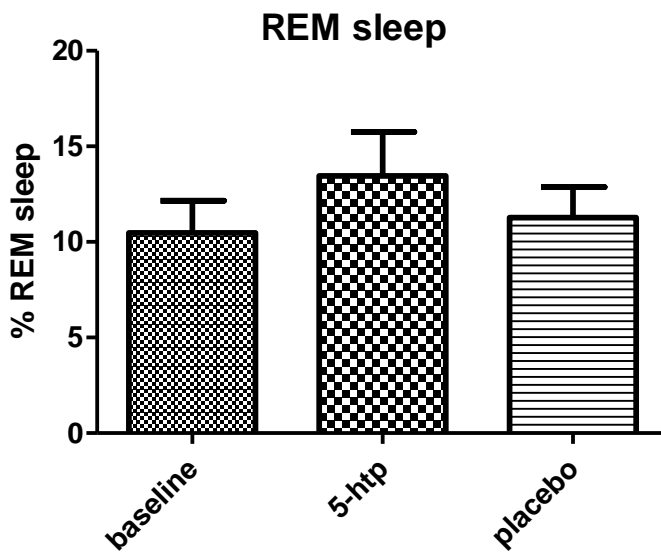
REM sleep

Polysomnographic sleep data : % REM sleep (primary outcome). One way anova (p=0.4; F=0.8)
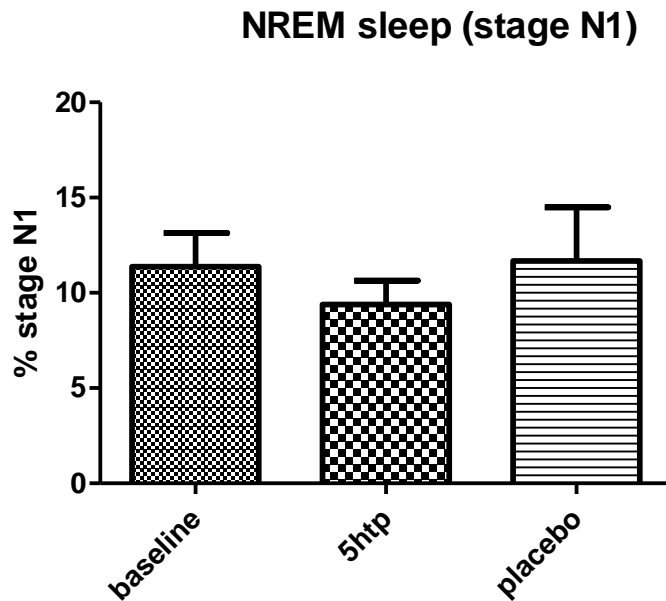
Fig.5
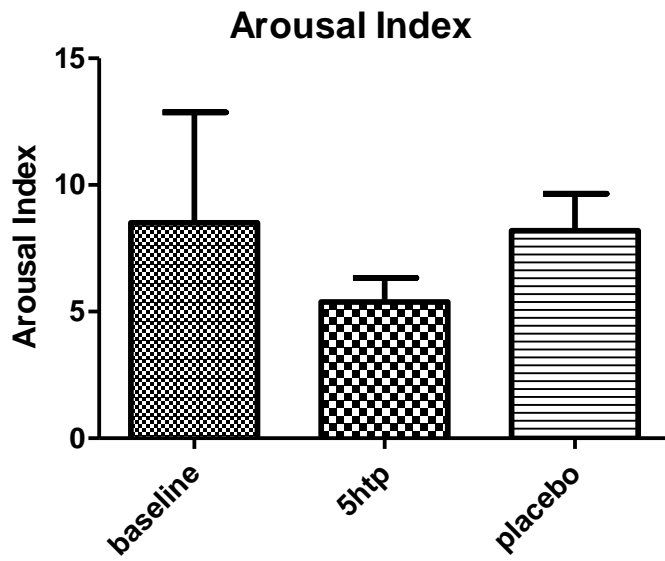
Arousal Index

Polysomnographic sleep data : Arousal index (primary outcome). One way anova (p=0.6; F=0.4)

Fig.6

Wake time after sleep onset
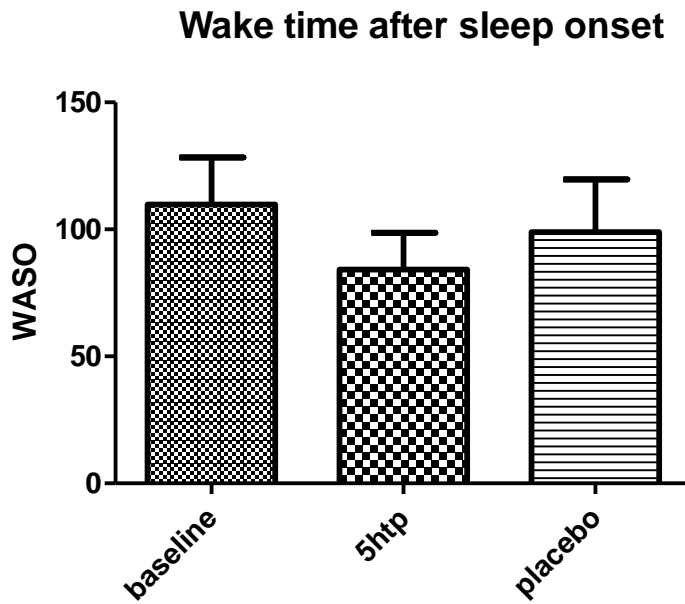
Polysomnographic sleep data : Wake time after sleep onset (WASO) (primary outcome). One way anova (p = 0.5; F=0.6)

Fig.7

NREM sleep (stage N1)

Polysomnographic sleep data : % stage N1 (NREM) (primary outcome). One way anova (*p*=0.5; F=0.5)

6.1.2 Depression

The two way repeated measure Anova analysis across all time points on BDI-II scores revealed no significant main effect for treatment (F=0.70; *p*=0.43) but revealed significant effect for time (F=3.75; *p*=0.01) (see Fig.8).

Treatment with 50 mg of 5-HTP significantly improved depression symptoms as rated by $HRDS_{21}$ scores compared to placebo. The two way Anova analysis revealed a trend (F=4.19; *p*=0.05) to significant effect by the treatment on $HRDS_{21}$ scores. Post-Hoc Bonferroni revealed significant difference between 5-HTP and placebo (t=3.10; $p < 0.05$) in the Part 2 (see Fig 9).

Unpaired t-test was performed to compare the mean $HRDS_{21}$ scores between groups (arms) in different treatment conditions (5-HTP versus placebo). According to Anova results, $HRDS_{21}$ scores differed significantly between the placebo and 5-HTP condition in the Part II (t=2.24; df=15; *p*=0.04) (see Fig. 10).
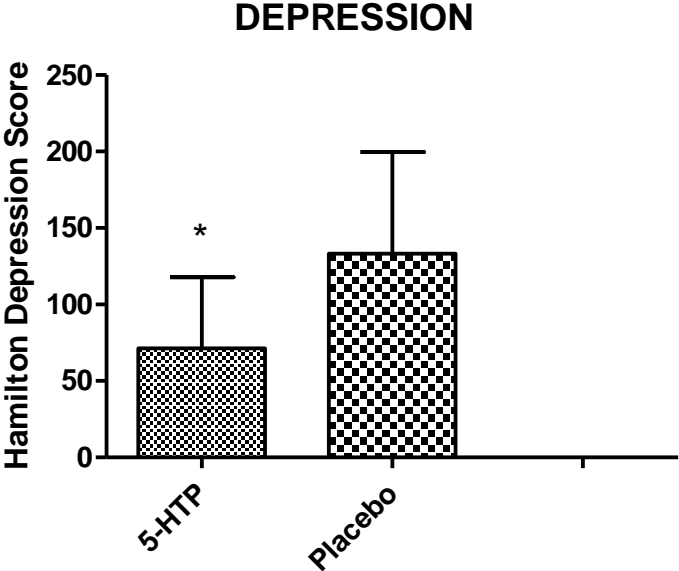
Fig.8
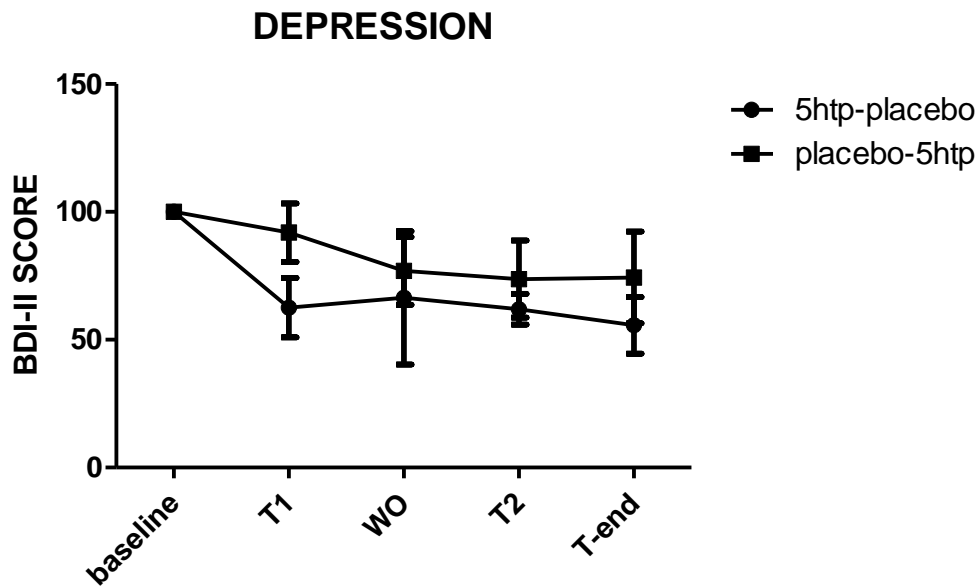
DEPRESSION

Depression rated using Beck Depression Inventory II (BDI-II) (primary outcome). Two way anova (treatment $p=0.4$, F=0.7; time $p=0.01$, F=3.75)

Fig.9
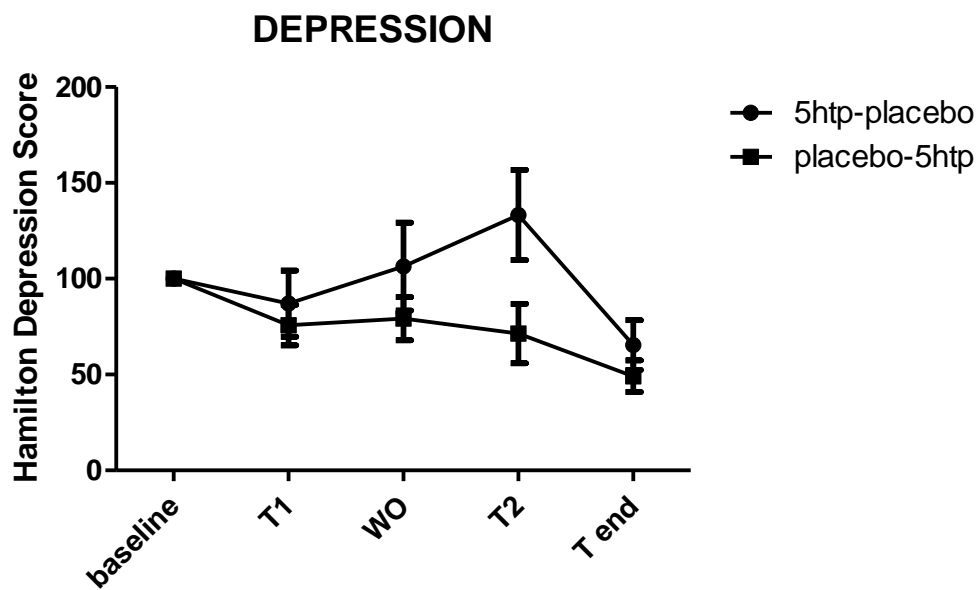
DEPRESSION

Depression rated using the Hamilton Depression Rating Scale 21-Item ($HDRS_{21}$) (primary outcome).
Two way anova (treatment $p=0.05$, F=4.19; time $p=0.005$, F= 4.05)

Fig. 10

DEPRESSION

Depression rated using the Hamilton Depression Rating Scale 21-Item (HDRS$_{21}$) (primary outcome).

Unpaired t-test (p=0.04; t=2.24; df=15)

6.1.3 Apathy

There were no significant effects of 5-HTP on apathy symptoms as rated by AS. The two way repeated measure Anova revealed no significant main effect neither for the treatments (5-HTP vs placebo) (F=0.26; p=0.62) nor for time (F=0.16; p=0.95) (see Fig. 11).
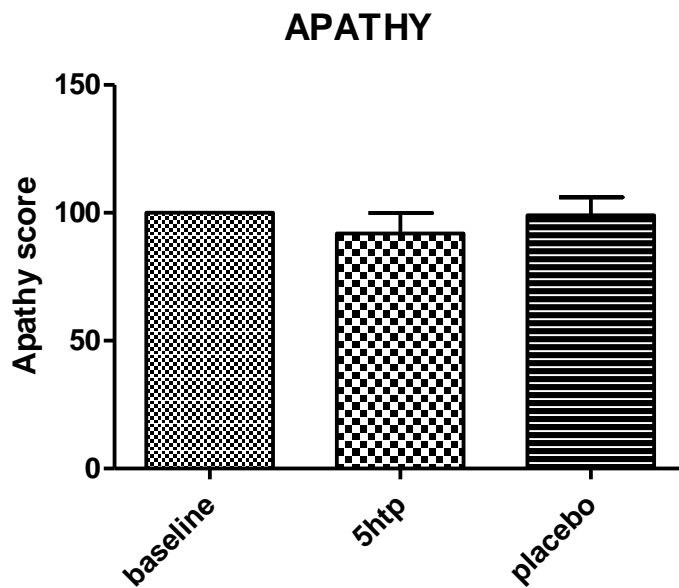
Compared to the baseline values, the mean AS scores were not improved by 5-HTP treatment in comparison to placebo (F=0.72; p=0.49; one-way Anova) (see Fig.12).

Fig. 11
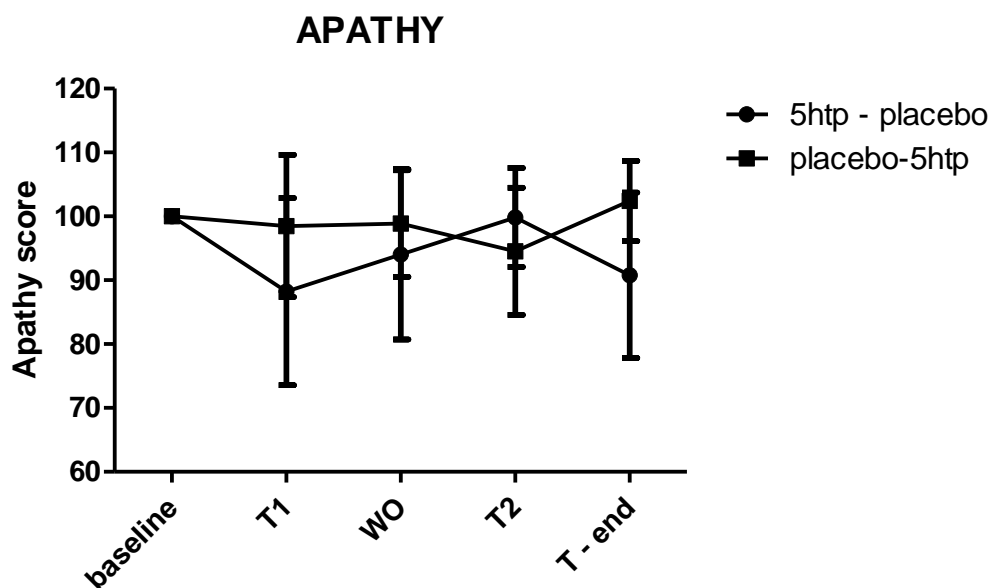
Apathy rated using the the Apathy Scale (AS) (primary outcome). Two way anova (treatment p=0.6, F=0.26; time p=0.9, F=0.16)

Fig. 12

APATHY

Apathy rated using the Apathy Scale (AS) (primary outcome). One way anova (p=0.49; F=0.72)

6.1.4 LID and motor fluctuations

Overall dyskinesia symptoms (UDysRS Part 1A/B score) revealed a significant improvement with 5-HTP compared to placebo.

Two way Anova didn't show significant main effect for treatment (5-HTP vs placebo) (F=1.82; p=0.21) and for time (F=2.41; p=0.06) (see Fig.13).

We performed one way Anova to compare UDyRS scores between baseline and 4 week treatment period with 5-HTP versus 4-week treatment period with placebo. Compared to placebo, there was a significant reduction of LIDs as measured by UDyRS (F=4.07; p=0.03). Post-Hoc Bonferroni's Multiple Comparison Test confirmed significant improvement on the UDyRS scores in patients treated with 5-HTP compared to placebo (t=2.8; $p < 0.05$) (see Fig.14).

We conducted a separate analysis of the UDyRS scores from dyskinetic PD subjects without RBD who took the daily dosage of 5-HTP in the morning. Analyses revealed that 5-HTP produced an improvement of dyskinesia symptoms without reaching statistical significance (one way Anova: p=0.06; F=3.46) (see Fig 15).

Patient-related motor fluctuations and dyskinesia severity as rated by UPDRS-IV showed significant differences between 5-HTP and placebo. The two way repeated measure Anova revealed no significant main effect for treatment (5-HTP vs placebo) (F=0.43; p=0.52) nor for time (F=2.15; p=0.09) (Fig.16).

One way Anova test was performed to compare UPDRS IV scores between baseline and 4 week treatment period with 5-HTP versus 4-week treatment period with placebo. Mean UPDRS IV score with 5-HTP was significantly reduced in comparison to placebo (F=3.4; p=0.04). Post-Hoc Bonferroni's Multiple Comparison Test confirmed significant improvement on the UPDRS IV scores in patients treated with 5-HTP compared to placebo (t=2.42; $p < 0.05$) (see Fig.17)

When the results of 5-HTP in Part I was compared to baseline, there was a significant difference in UPDRS-IV ratings (baseline: 8.6±3.4 versus 5-HTP: 6.0±2.3; paired t-test: t=3.34; df=6; p= 0,01) (see Fig.18). A separate analysis of UPDRS IV data from dyskinetic patients without RBD confirmed a significant improvement with 5-HTP compared to placebo (two way Anova: treatment p=0.05, F=6.23; time; p=0.13, F=2.0; paired t-test: t=3.30; df=3; p= 0,04) (Fig.19; 20).

There were no significant effects of 5-HTP on patient-related motor fluctuations severity as rated by WOQ-19 questionnaire. There were no significant differences in the 5-HTP treatment compared to placebo at any of the assessment time points.
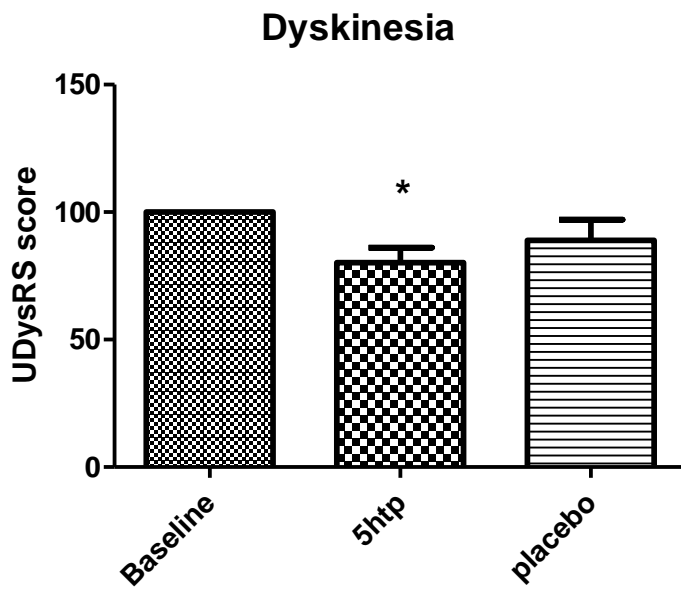
Fig. 13
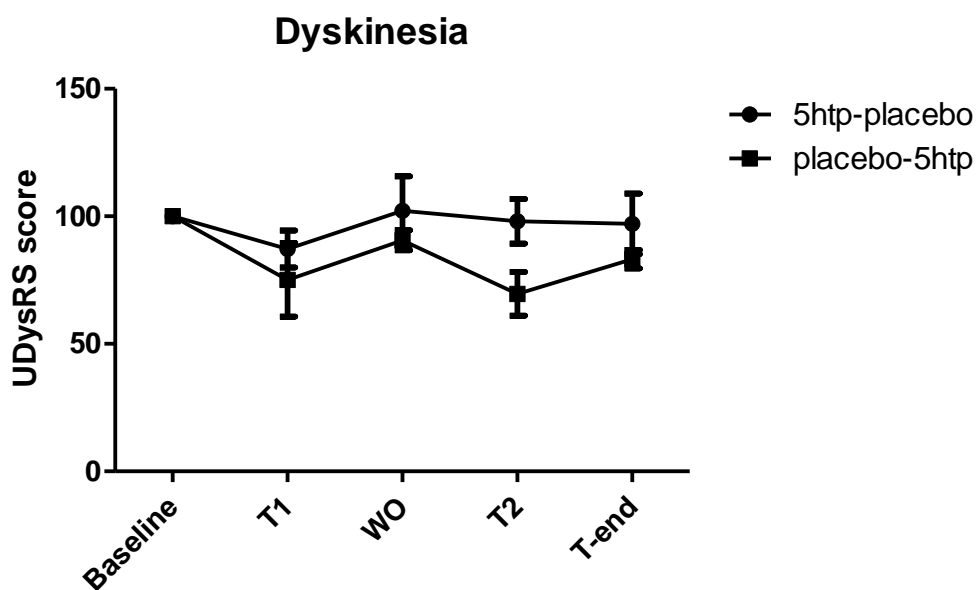
Dyskinesia severity rated using the Unified Dyskinesia Rating Scale (UDysRS) (primary outcome).
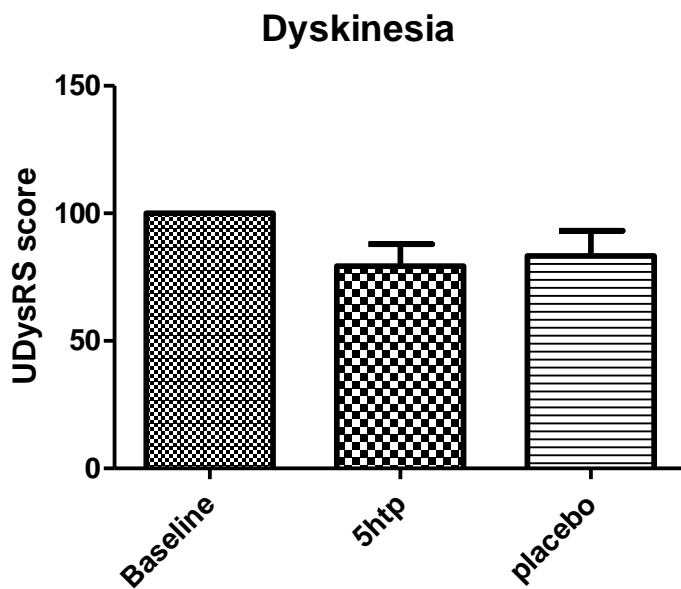
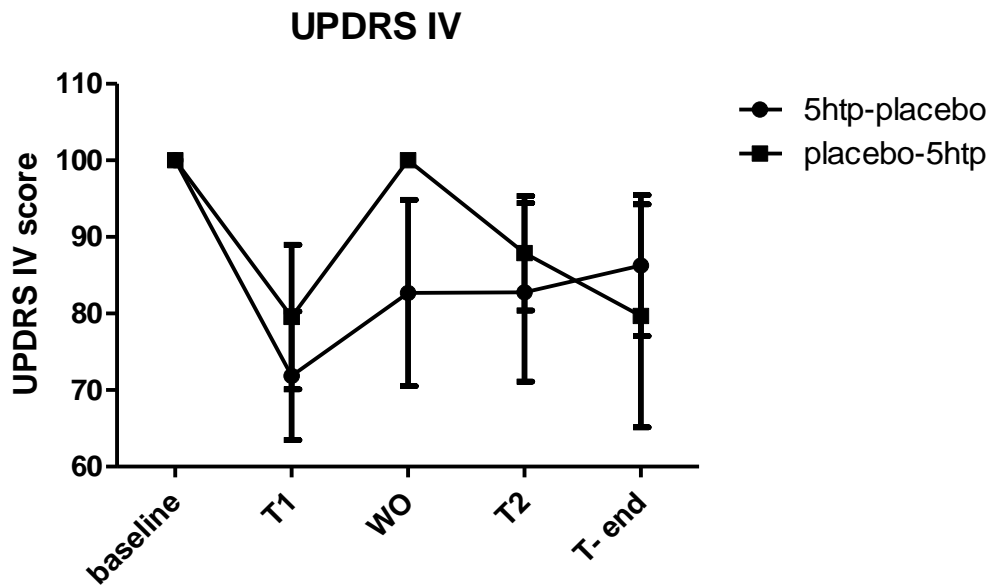Two way anova (treatment p=0.21, F=1.82; time p=0.06, F=2.41)

Fig.14

Dyskinesia

Dyskinesia severity rated using the Unified Dyskinesia Rating Scale (UDysRS) (primary outcome).
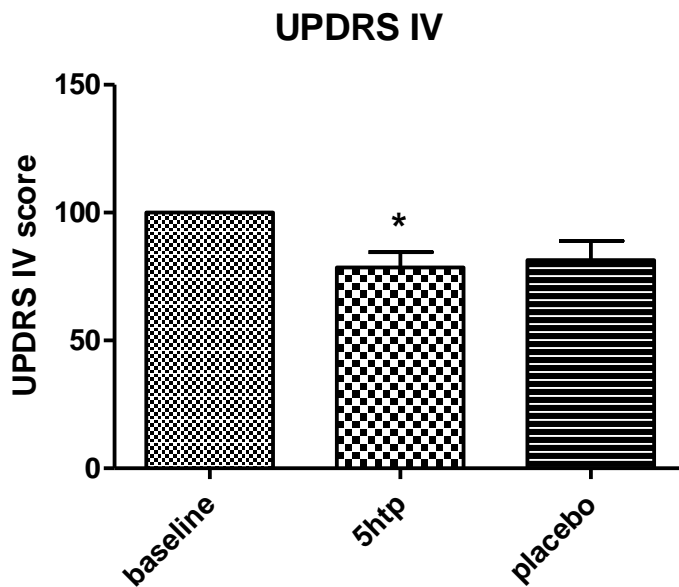One way anova (p=0.03; F=4.07)

Fig 15

Dyskinesia

Dyskinesia severity rated using the Unified Dyskinesia Rating Scale (UDysRS) (primary outcome).
A separate analysis from dyskinetic PD subjects without RBD. One way anova (p=0.06; F=3.46)
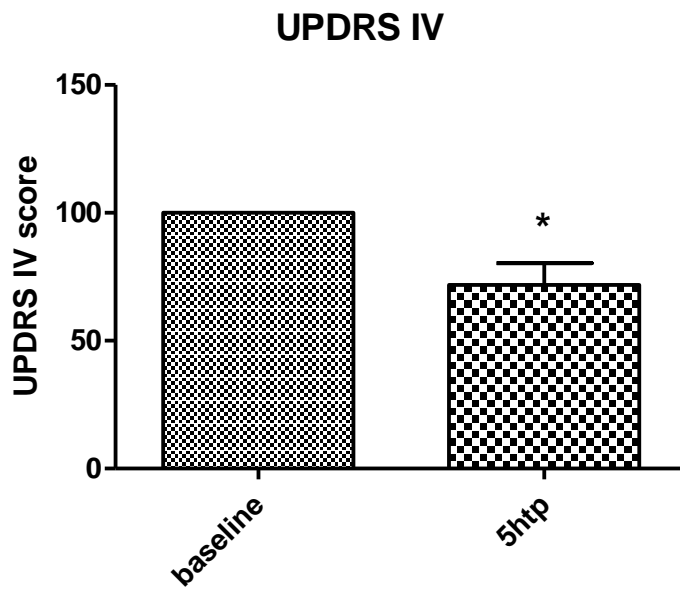
Fig.16

UPDRS IV

Dyskinesia and motor fluctuactions severity rated using the Unified Parkinson Disease Rating Scale Part IV (UPDRS IV) (primary outcome). Two way anova (treatment $p=0.52$, F=0.43; time; $p=0.09$, F=2.15)
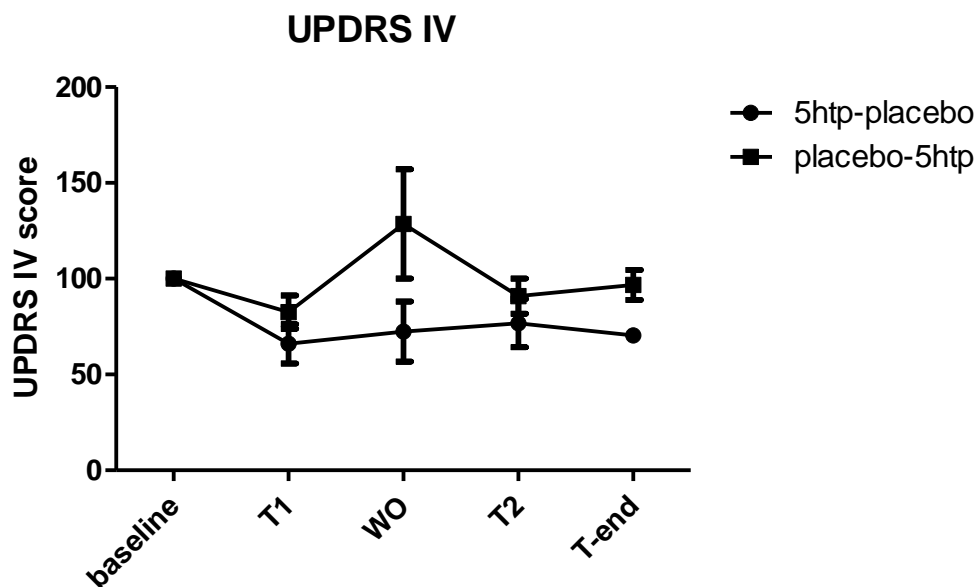
Fig.17

UPDRS IV

Dyskinesia and motor fluctuactions severity rated using the Unified Parkinson Disease Rating Scale Part IV (UPDRS IV) (primary outcome). One way anova (F=3.4; $p=0.04$)
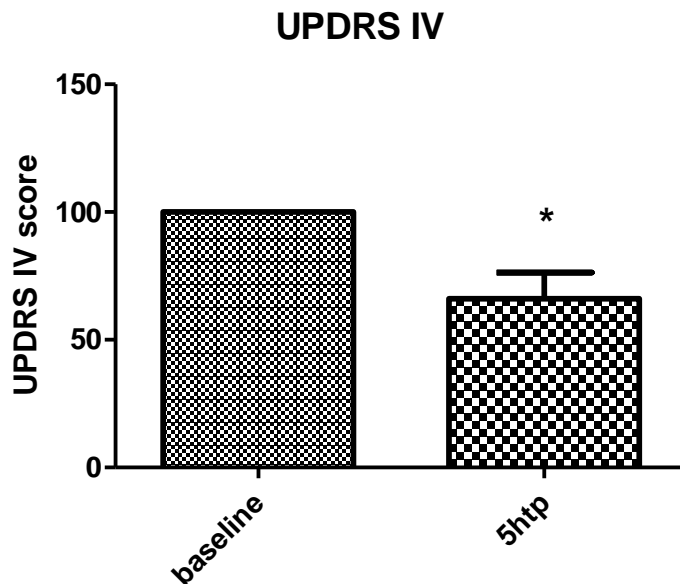
Fig. 18

UPDRS IV

Dyskinesia and motor fluctuactions severity rated using the Unified Parkinson Disease Rating Scale Part IV (UPDRS IV) (primary outcome). Paired t-test (t=3.349; df=6; p= 0,01)

Fig.19

UPDRS IV

Dyskinesia and motor fluctuactions severity rated using the Unified Parkinson Disease Rating Scale Part IV (UPDRS IV) (primary outcome). A separate analysis from dyskinetic PD subjects without RBD. Two way anova (treatment p=0.05, F=6.23; time; p=0.13, F=2.0).

Fig.20

Dyskinesia and motor fluctuactions severity rated using the Unified Parkinson Disease Rating Scale Part IV (UPDRS IV) (primary outcome). A separate analysis from dyskinetic PD subjects without RBD. Paired t-test (t=3.30; df=3; *p*= 0,04).

6.2 Changes in secondary outcomes:

6.2.1 Overall functional status

The two way repeated measure Anova on UPDRS Part I revealed no significant main effect for treatment (F=0.18; p= 0.67) and time (F=1.75; *p* = 0.14) (see Fig. 21).

Compared to baseline, 5-HTP and placebo reduced significantly the mean UPDRS Part I score (one way Anova: p=0.0001; F=20.35). Post-Hoc Bonferroni's Multiple Comparison Test confirmed significant improvement on the UPDRS Part I scores in patients treated with 5-HTP and placebo compared to baseline (baseline vs 5-HTP *p*<0.01, t=3.8; baseline vs placebo *p*<0.001, t=6.3) (see Fig.22).

The two way repeated measure Anova revealed significant interactions between time and treatment for the UPDRS Part II (F=2.75; p=0.03). Post-Hoc Bonferroni analysis revealed significant differences between treatment period with 5 HTP and placebo during the Part II ($p<0.05$) (see Fig. 23). One way Anova analysis didn't show any significant difference between the two treatments (see Fig. 24)

The two way repeated measure Anova revealed no significant main effect for treatment (F=0.45; p= 0.51) and for time for the UPDRS Part III (F=0.84; p = 0.50) (see Fig.25).

One way Anova analysis showed that the improvement in motor scores from baseline as measured by UPDRS Part III was significantly greater in patients receiving 5-HTP compared to placebo (baseline vs 5-HTP: t=2,426; p < 0.05; Post-Hoc Bonferroni analysis)(see Fig.26).
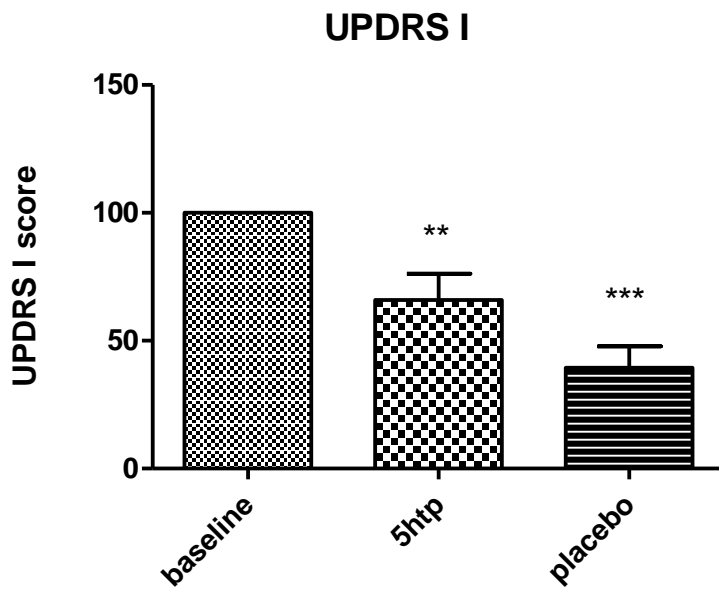
No significant effect for treatment (5-HTP/placebo) but significant time effect was assessed by two way Anova analysis on Total UPDRS (F=2.83; p = 0.02; see Fig 27).

Fig. 21
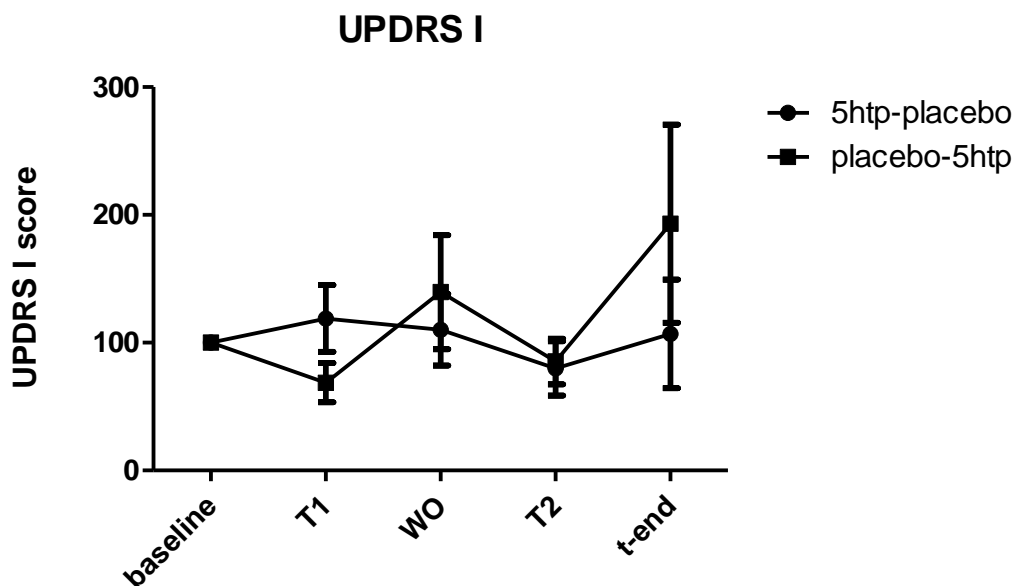
Mentation, mood and behavior rated using the Unified Parkinson Disease Rating Scale Part I (UPDRS I) (secondary outcome). Two way anova (treatment p= 0.67, F=0.18; time p = 0.14, F=1.75)

Fig. 22

UPDRS I

Mentation, mood and behavior rated using the Unified Parkinson Disease Rating Scale Part I (UPDRS I) (secondary outcome). One way anova ($p=0.00$; F=20.35)

Fig. 23

UPDRS II

Activities of daily living rated using the Unified Parkinson Disease Rating Scale Part II (UPDRS II) (secondary outcome). Two way anova (treatment $p= 0.34$, F=0.94; time $p = 0.71$, F=0.52; interaction $p= 0.03$, F=2.75)

Fig.24

UPDRS II

Activities of daily living rated using the Unified Parkinson Disease Rating Scale Part II (UPDRS II) (secondary outcome). One way anova ($p=0.38$; F=0.96)
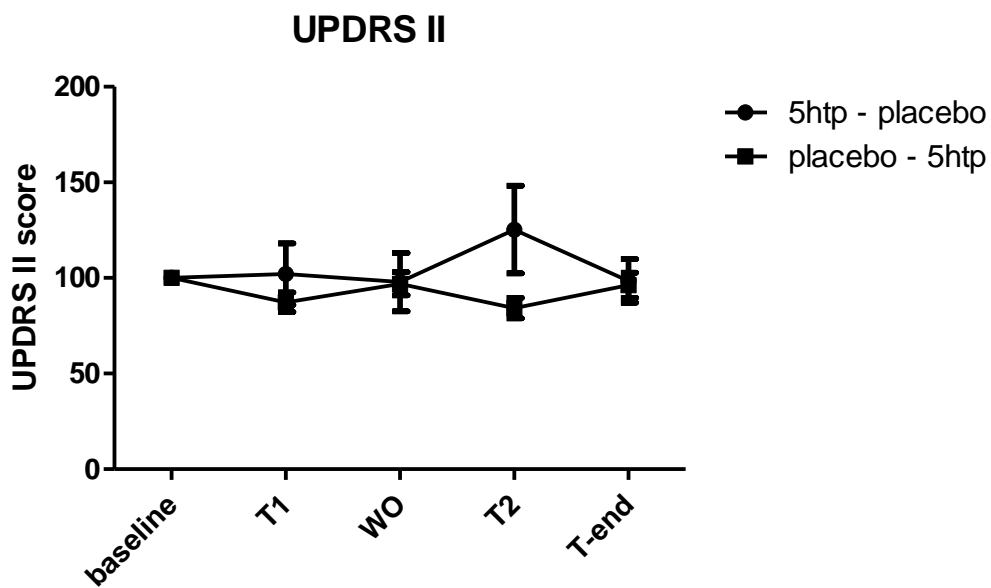
Fig.25

UPDRS III

Motor examination rated using the Unified Parkinson Disease Rating Scale Part III (UPDRS III) (secondary outcome). Two way anova (treatment $p= 0.51$, F=0.45; time $p = 0.50$, F=0.84)
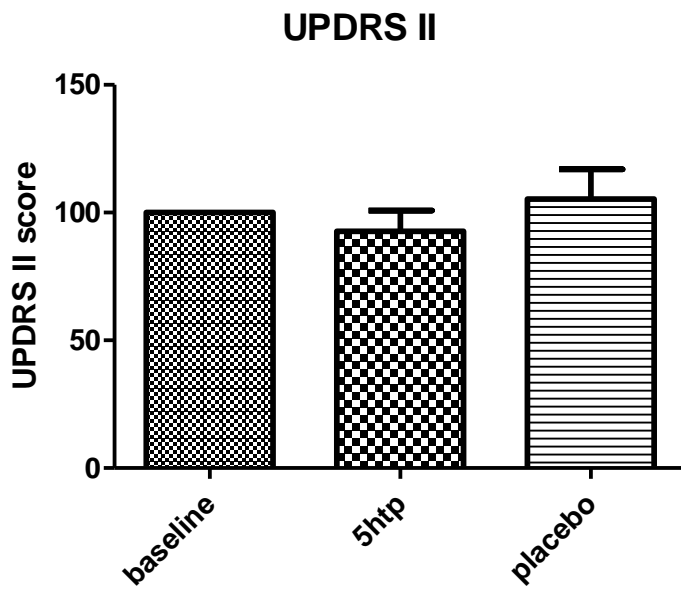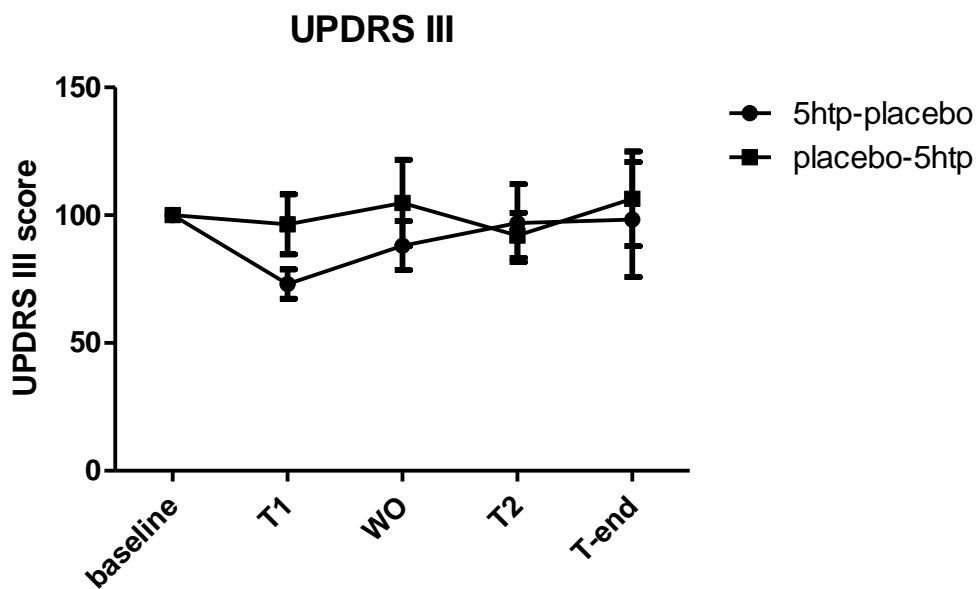
Fig.26
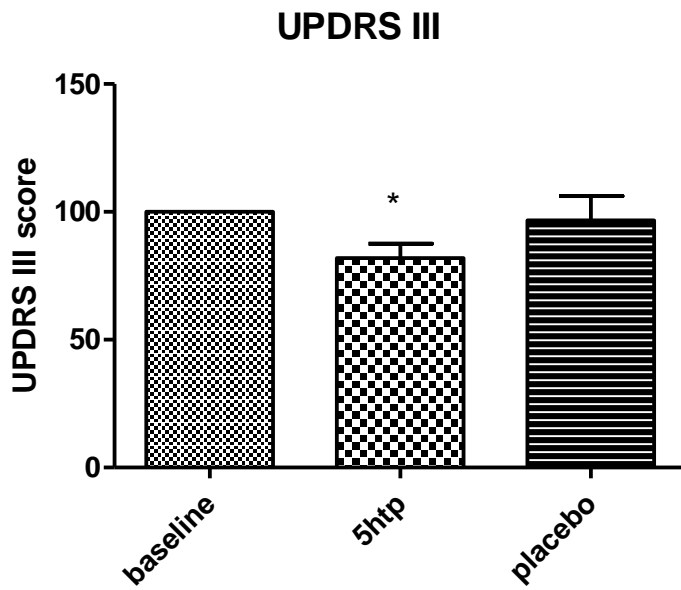
UPDRS III

Motor examination rated using the Unified Parkinson Disease Rating Scale Part III (UPDRS III) (secondary outcome). One way anova (p=0.05, F=3.33; baseline vs 5-HTP: t=2,426; p < 0.05; Post-Hoc Bonferroni analysis)

Fig.27

Total UPDRS
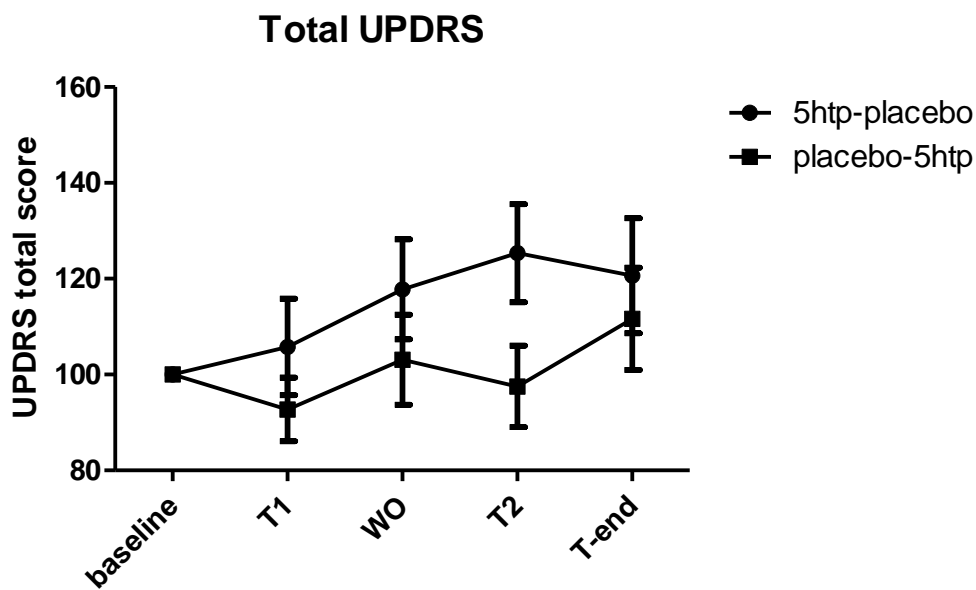
Overall functional status rated using the total score of all parts of the Unified Parkinson Disease Rating Scale (secondary outcome). Two way anova (treatment p=0.23, F=1.50; time p=0.02, F=2.83)

6.2.2 Sleep variable

No significant effect for treatment (5-HTP/placebo) but significant time effect was assessed by two way Anova analysis on RSS scores (F=5.20; p=0.003, see Fig 28).

Fig. 28
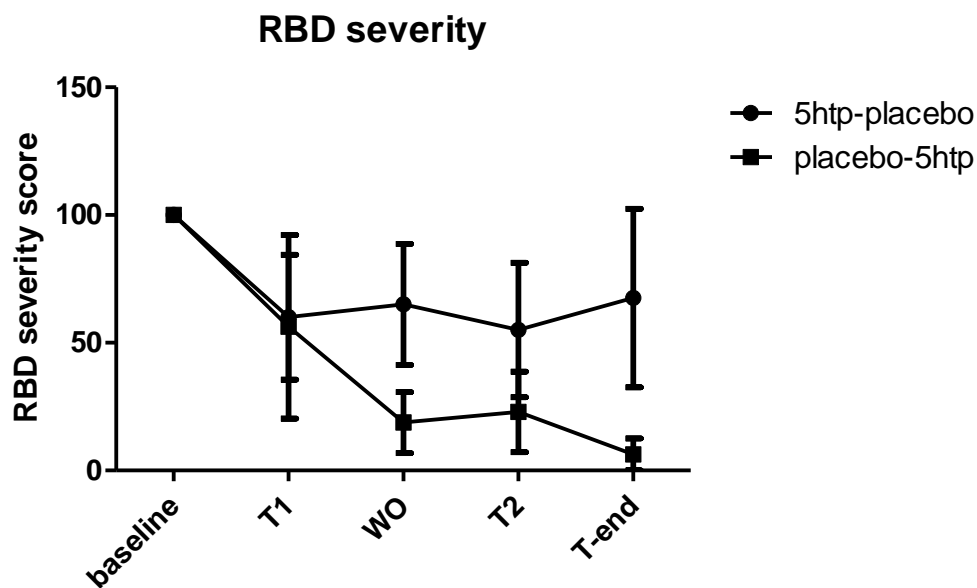
RBD severity

RBD severity rated using the RBD Severity Scale (RSS) (secondary outcome). Two way anova (treatment p=0.21, F=1.94; time p=0.003, F= 5.20)

6.2.3 Home dyskinesia diary data

A total of 6 patients completed the self-reported 24-hour home dyskinesia diaries. As shown in Fig. 29, there was a trend towards a decrease of the time spent in "on" with moderate dyskinesia with 5-HTP treatment compared to placebo (17.6±17.7 h versus 14.5±14 h), although this did not reach statistical significance (t=0.33; p>0.05).

As shown in Fig. 30 there was a trend towards a decrease of the time spent in "mild" and "moderate" rigidity with 5-HTP treatment compared to placebo (respectively 60.3±38.7 h versus 58.1±20.3 h; 38.6±18.8 h versus 34.8±24.6 h), although this did not reach statistical significance (respectively t=0.14; t=0.25; p>0.05).

Fig.29

Dyskinesia home diary data
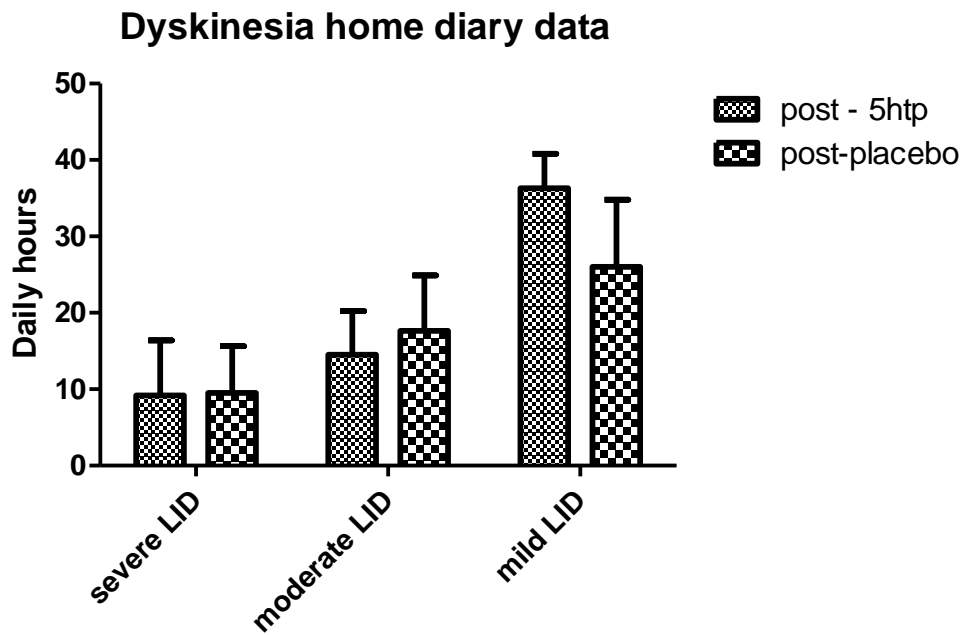
Total daily hours in severe, moderate and mild LID evaluated from patient diaries (secondary endpoint).

Two way anova (severity $p=0.005$, F=6.90; treatment $p=0.73$, F=0.12)

Fig. 30

Rigidity home diary data
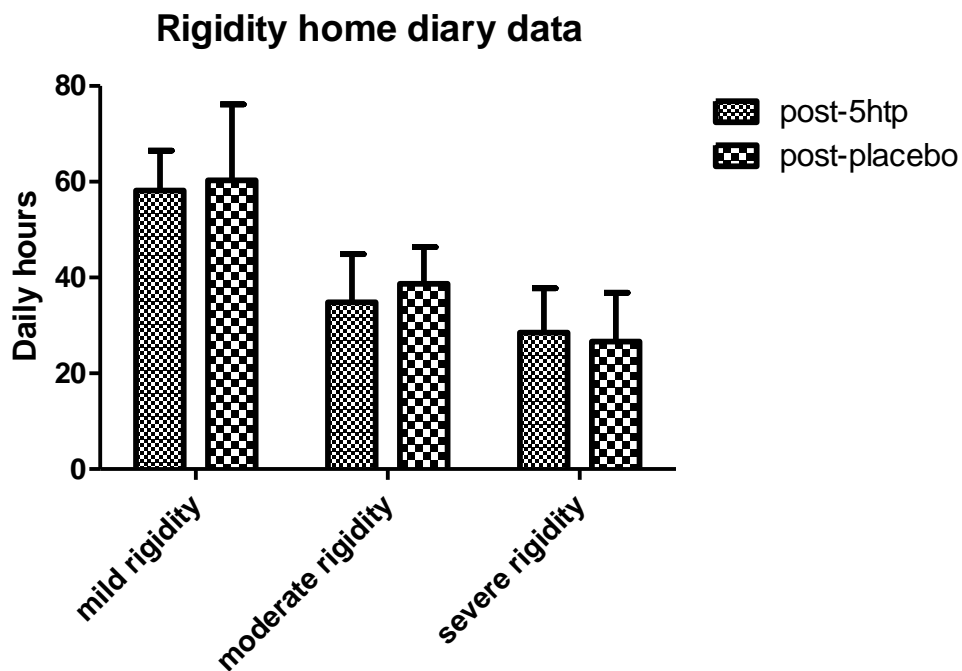
Total daily hours in severe, moderate and mild rigidity evaluated from patient diaries (secondary endpoint).

Two way anova (severity $p=0.01$, F=5.09; treatment $p=0.88$, F=0.02)

The sleep, motor complications and neuropsychiatric outcome measures at baseline and at the end of 5-HTP and placebo study arms are presented respectively in Table 2; 3 and 4.

Table 2

Study outcome measures: sleep variables				p value		
Variables	Baseline	5-HTP	Placebo	Baseline vs 5-HTP vs Placebo	Baseline vs 5-HTP	5-HTP vs Placebo
% Any EMGChin	64.0±29.0	80.3±20.2	74.4±22.8	*ns*	*ns*	*ns*
%Any EMG Chin+FDS (3 sec)	68.2±26.8	81.5±18.8	77.2±20.4	*ns*	*ns*	*ns*
% Any EMG Chin+FDS (30 sec)	73.2±32.8	87.3±15.9	88.4±15.5	*ns*	*ns*	*ns*
TST	378.1±84.3	346±76.2	323.5±85.3	.03*	*ns*	*ns*
SE	72.8±16.7	70.1±12.2	72±18.7	*ns*	*ns*	*ns*
WASO	109.8±73.7	84.1±58.3	98.9±83	*ns*	*ns*	*ns*
Arousal index (n/h)	6.6±8.5	5.4±3.2	6.7±3.7	*ns*	*ns*	*ns*
Stage N1 (%)	11.4±7	9.4±5	11.7±11.3	*ns*	*ns*	*ns*
Stage N2 (%)	48.8±11.8	54.3±12.3	52±8.1	*ns*	*ns*	*ns*
Stage N3 (%)	29.3±12.3	22.9±9.5	25±8	*ns*	.04 ˆ	*ns*
Stage REM sleep (%)	10.4±6.8	13.4±9.2	11.3±6.4	*ns*	*ns*	*ns*
REM sleep periods (n)	2.8±1.2	2.5±0.7	2.4±1.2	*ns*	*ns*	*ns*
RSS	4.7±3.4	2.2±2.9	2.5±3.1	*ns*	*ns*	*ns*

Data are presented as mean and standard deviation (S.D);

Abbreviations: Any: phasic and/or tonic; EMG: electromyographic; FDS: flexor digitorum superficialis; RSS, RBD Severity Scale; TST: Total sleep time; SE: Sleep efficiency; WASO: Wake time after sleep onset; *ns* : not significant; *Repeated measures ANOVA; ˆPaired t-test; ˜Unpaired t-test.

Table 3

Study outcome measures: LIDs and Motor fluctuactions				*p* value		
Variables	Baseline	5-HTP	Placebo	Baseline vs 5-HTP vs Placebo	Baseline vs 5-HTP	5-HTP vs Placebo
UDyRS (Part 1A/B score)	22.9±8.6	17.6±5.6	19.7±8.8	.03*	*ns*	*ns*
UDyRS (Part 1A/B score) **	22.1±9.8	16.5±6.3	17±6	*ns*	*ns*	*ns*
UPDRS IV	8.3±3.2	6.4±2.6	6.7±2.8	.04*	.01ˆ	*ns*
UPDRS IV **	9.9±3.3	7.4±2.7	7.7±2.7	*ns*	.04ˆ	*ns*
WOQ-19	7.5±4.6	6.2±1.9	5.7±2.0	*ns*	*ns*	*ns*

Data are presented as mean and standard deviation (S.D);

Abbreviations: UPDRS, Unified Parkinson's Disease Rating Scale; Unified Dyskinesia Rating Scale; WOQ-19, Wearing-off Questionnaire 19 items; *ns* : not significant;

**Dyskinetic subjects without RBD; *Repeated measures ANOVA; ˆPaired t-test; ˜Unpaired t-test.

Table 4

Study outcome measures: depression and apathy				*p* value		
Variables	Baseline	5-HTP	Placebo	Baseline vs 5-HTP vs Placebo	Baseline vs 5-HTP	5-HTP vs Placebo
BDI-II	20.4±8.0	13.1±5.2	16.5±7.4	*ns*	*ns*	*ns*
HDRS$_{21}$	15.0±5.7	11.4±7.7	14.1±6.4	*ns*	*ns*	.04˜
AS	17.4±2.7	16.0±5.6	17.0±3.6	*ns*	*ns*	*ns*

Data are presented as mean and standard deviation (S.D);

Abbreviations: BDI-II, Beck Depression Inventory-II; HDRS, Hamilton Depression Rating Score 21 items; AS, Apathy Scale; *ns* : not significant; *Repeated measures ANOVA;

ˆPaired t-test; ˜Unpaired t-test.

6.3 Side effects

The 5-HTP was well tolerated through successive cycles of therapy. No 5-HTP-related side effects were reported. The headache, fever, and constipation reported in other studies were not observed. No patient withdrew from the trial because of adverse events.

Chapter 7: Discussion and Conclusions

To the best of our knowledge this is the first randomized, double-blind, placebo-controlled, crossover clinical treatment study to evaluate the efficacy of the 5-HTP on RBD, apathy, depression and levodopa - induce motor complications.

We have found a reduction of the percentage of time in stage N1 and N3 with an increase in total percentage of REM sleep from treatment with 50 mg 5-HTP in subjects with PD.

Particularly, the increase in total percentage of REM sleep was not associated with the increase of the percentage of RBD episodes as rated by PSG and RSS assessments.

In that study, a non-significant trend for decreased arousal index and the amount of time spent awake after sleep was found in subjects who received 5-HTP compared to placebo, contribuiting to ameliorate the global sleep quality.

These results are in line with previous studies in the literature. The 5-HTP has been shown to improve sleep quality by increasing REM sleep [183-185]. However these studies have not been conducted with PD patients. To the best of our knowledge these results have not been replicated yet in PD patients.

Serotonin acts as a positive modulator for melatonin synthesis: the rate of pineal melatonin synthesis is dependent on the free cytoplasm pool of serotonin in pinealocytes, and the drug-induced elevation of this pool stimulates melatonin formation and increases circulating melatonin levels [201]. The increase of the nocturnal serotonin levels would enhance the nocturnal production of melatonin. Indeed, the melatonin is biosynthesized from serotonin through the action of arylalkylamine N-acetyltransferase (serotonin N-acetyltransferase) and, it seems that serotonin N-acetyltransferase activity is stimulated by the serotonin via the 5-HT2 receptor [202; 203].

REM sleep is associated with individual melatonin excretion levels. Exogenous melatonin increases REM sleep percentage to normal levels in patients with reduced REM sleep duration and re-organizes REM sleep episode length during night-time sleep [204]. Among all sleep stages, REM sleep is the most circadian-dependent. In fact, REM sleep duration, latency, and continuity are under strong circadian control [205]. Melatonin deficit leads to instability in the circadian timing system, resulting in reduced REM sleep during nighttime sleep [206].

On the other hand, most antidepressants inhibit REM sleep in animals and humans. In particular, SSRIs, such as citalopram and paroxetine are potent inhibitors of REM sleep when administered acutely, sub-chronically or chronically [207].

According to these scientific premises we hypothesized that 5-HTP may have contribuited to increase the total percentage of REM sleep in our PD patients through the increase of melatonin levels.

We report the efficacy of the 5-HTP on depressive symptoms in patients with PD; scores on both the primary (BDI-II and $HDRS_{21}$) outcome measures showed substantially greater improvement with 5-HTP than with placebo but the improvement was statistically significant only on the $HDRS_{21}$.

These results are in line with the literature. The pathophysiological mechanisms involved in depressive symptomatology in PD remain unclear, but serotonergic dysfunction has been demonstrated to be involved in mood disorders in PD and the raphe nuclei, as well as hippocampus and prefrontal cortex, appear to be the primary sites affected.

CSF measurements in vivo have shown reduced serotonin metabolite (5-HIAA) levels in depressed patients with PD [108; 116]. Using 18F-MPPF PET, Ballanger and colleagues have found decreased 5-HT1A receptor availability in limbic regions including the right insula, the left hippocampus, the orbitofrontal region and the uncus in depressed PD patients [117].

These findings are in agreement with post-mortem evidence [118] and support the hypothesis that reductions in postsynaptic 5-HT1A receptors availability could be an underlying mechanism for the development of depression in PD.

Several PET studies using 11C-DASB, have reported relative increases of SERT binding in limbic structures, caudal raphe nuclei, posterior cingulate, dorsolateral and prefrontal cortices of depressed PD patients compared to matched-PD patients without depression [119;120].

As the disease progresses, Lewy bodies occur within the rostral raphe, thalamus, and limbic and cortical regions [26] which may result in the mediating of mood disturbance in advanced PD.

Interestingly, PD progression results in degeneration of the dorsal raphe nuclei neurons which precedes the loss of nigral dopamine neurons, although not to the same extent [26]. In fact, PD patients with depression have been shown to have more severe dorsal raphe nuclei cell loss compared to PD patients without depression [208]. Therefore, it is conceivable that levodopa-induced serotonin deficits may further exacerbate existing behavioral impairments in PD patients. In support of this concept, affective disorders are not improved by levodopa treatment [209], and may in fact can be worsened by chronic levodopa therapy.

Dopamine has been shown to damage cellular proteins in serotonergic neurons. Dopamine has long been known to be a potent oxidant [210], as unsequestered dopamine can serve as a pro-oxidant when it auto-oxidizes into quinone species. Tryptophan hydroxylase (TPH), the rate limiting

enzyme in serotonin production, is inactivated by dopamine-quinones [211]. Additionally, in vitro and in vivo studies have demonstrated that dopamine can produce cell death in serotonergic cell culture via reactive oxygen species production resulting from the synthesis and degradation of dopamine after exogenously applied levodopa [212; 213]. In addition to levodopa damage to serotonergic neurons at the cell body level, levodopa also exerts effects on serotonin neurotransmitter production and release at the level of serotonergic axon terminal throughout the brain. Several reports indicate that both acute, as well as chronic, levodopa can result in serotonin deficits. The acute exogenous levodopa may impair 5-HT function by inhibiting tryptophan hydroxylase and by competing for conversion via aromatic amino acid decarboxylase (AADC) [109; 110; 111; 214; 215].

In addition to decreases in serotonergic tissue content within several brain areas, extracellular serotonin was also decreased after chronic levodopa in the striatum, substantia nigra pars reticulata, hippocampus, and prefrontal cortex. Furthermore, they also found that acute levodopa treatment resulted in significantly less dopamine release in these regions, suggesting that these 5-HT terminals that normally release dopamine after levodopa are compromised [111]. Overall, there is substantial pre-clinical evidence for the negative impact of chronic levodopa on the serotonergic system.

Deficits in serotonin caused by levodopa administration may dysregulate crucial brain areas for cognitive and affective behaviors such as the amygdala, striatum, hippocampus, dorsal raphe nucleus and prefrontal cortex. Deficits in these brain regions could impact affective behavior such as depression.

Moreover, the conversion of levodopa into dopamine by serotonergic neurons has been suggested to result in serotonin depletion by competing for storage into serotonergic vesicles [111-113].

These findings suggest that a combined effect of serotonergic terminal loss together with inappropriate upregulation of 5-HTT functions and chronic levodopa treatment may contribute to the development of depressive symptoms in PD.

A possible strategy to counteract the levodopa-induced reduction of extracellular serotonin levels may be represented by the administration of the serotonin precursor 5-HTP. Indeed, 5-HTP has been shown to increase central serotonin levels when given with a peripheral decarboxylase inhibitor [216]. We speculated that 5-HTP administration may be beneficial through two possible mechanisms. First, by increasing cytoplasmic serotonin synthesis and consequent availability in the synaptic cleft; second, competing with levodopa-derived DA for storage into the serotonin synaptic vesicles, therefore, reducing serotonin-dependent DA release leading to less oxidative stress produced by levodopa-induced supraphysiologic concentrations of dopamine.

No statistically significant improvement in apathy symptoms as rated by the AS were observed with 5-HTP versus placebo.

Convergent data suggest that apathy is part of a hypodopaminergic syndrome related to mesolimbic dopaminergic dysfunctions. Of particular interest are neuroimaging reports in PD of a dysfunction of dopaminergic transmission in the mesocorticolimbic pathway that might underlie apathetic behaviour. In this regard, PET studies with the dopamine D2 and D3 receptor antagonist [^{11}C]raclopride have shown several differences in dopaminergic binding and transmission between PD patients with apathy and those without apathy [144]. Postoperative apathy is a frequent observation after STN-DBS in PD [217]. The isolated apathy that occurs with chronic STN-DBS during the first postoperative year in relatively young patients with PD, with predominant degeneration of midbrain dopaminergic neurons in the substantia nigra and ventral tegmental area (but without much cortical synucleinopathy), has been proposed as a model of fairly pure dopamine-dependent apathy. This model points to the importance of dopamine in both cognitive and emotional aspects of motivated human behaviour and provides a rationale for dopaminergic treatment in the management of apathy in PD. Recents exploratory studies showed that the dopamine D2 and D3 receptor agonists significantly improved apathy in PD patients presenting apathy after STN-DBS [151; 218].

To date, the use of antidepressants for apathy in PD is controversial.

We failed to find significant improvements on apathetic symptoms by the treatment with 5-HTP compared to placebo. Our results are in line with the prevalent dopaminergic impairment in PD patients with apathy. Hence, it may be that the effects of simply enhancing the serotonergic system by increasing serotonin synthesis are not enough to treat apathy symptoms in patients with PD.

This study evaluated 5-HTP as a potential adjunctive therapy of LIDs and motor fluctuactions in patients with PD.

The major finding of this study was that patients receiving 5-HTP at 50 mg experienced a significant reduction of LIDs, measured by either UDyRS and UPDRS Part IV, compared to those treated with placebo. On the contrary, we didn't find significant effects of 5-HTP on wearing-off (WO) phenomenon as rated by WOQ-19 questionnaire.

In PD, conversion sites for levodopa to dopamine diminish progressively. Several abnormal compensatory cellular and receptor complex alterations develop to counteract the dopamine deficiency. These alterations, together with levodopa treatment, result in WO of medication effects and development of LIDs. In advanced stage of dopaminergic cell loss, the remaining serotonergic neurons in the basal ganglia complex can specifically take up levodopa and convert it to dopamine

[50-52]. However, serotonin neurons lack a feed-back control mechanism able to regulate the synaptic levels of DA, which is provided in DA neurons by the DA D2 autoreceptor.

In turn, uncontrolled release of DA from serotonin neurons cooperates with levodopa administration to produce pulsatile stimulation of postsynaptic DA receptors [54; 112]. In particular, uncontrolled stimulation of supersensitized dopamine D1 receptors in the direct striatonigral pathway are thought to mediate LIDs. Accordingly, removal of the forebrain serotonin innervation by toxin lesion, or pharmacological silencing of serotonin neurons activity, suppressed LID both in the rat and monkey models of PD [54; 55].

Serotonin release is regulated by somatodendritic 5-HT1A receptors and nerve terminal 5-HT1B receptors. In animal models of PD, 5-HT1A and 5-HT1B receptor agonists act synergistically and can completely eliminate LIDs [54]. Therefore, serotonin 5-HT1 receptor agonists hold promise for the treatment of dyskinesia in PD patients. However, pharmacological silencing of serotonin neurons raises concern for possible negative effects on depressive symptoms. Treatment with selective serotonin autoreceptor agonists would not only reduce the serotonin-dependent DA release, but it is also expected to dampen the synaptic release of serotonin.

We hypothesized that 5-HTP may exert antidyskinetic effect by two possible mechanisms. First, by increasing cytoplasmic serotonin synthesis, which may compete with levodopa-derived DA for storage into the serotonin synaptic vesicles, therefore, reducing serotonin dependent DA release; second, leading to increased activation of serotonin autoreceptors, thus, mimicking the effect of selective serotonin 5-HT1 receptor agonists.

Importantly, 5-HTP reduced LIDs without impairing motor function and/or altering normal motor responses to levodopa as evaluated by UPDRS Part III.

These results showed that 5-HTP supplementation may be effective in reducing depressive symptoms and levodopa-induced motor complications in patients with PD. However, larger studies would be required to confirm this observation. Moreover, our data suggest the potential efficacy of the 5-HTP on the overall functional status as rated by the UPDRS I-II-III, but this requires further investigation. Notably, motor examination scores (UPDRS-III) significantly reduced during 5-HTP treatment demonstrating that there is no deterioration of the normal treatment effect of levodopa by 5-HTP co-treatment.

The main limitation of our study is the low dosage (50 mg/day) of 5-HTP. The possibility that the dose of 5-HTP used in this study was too low to exert a full biological effect is proven by trends to improvement in several clinical assessments without reaching statistical significance.

All patients underwent one full-night attended video-PSG recording in sleep laboratory with digital polysomnography. The fact that the first PSG was done in the sleep center and the subsequent ones were done in patients' homes may have caused heterogeneity of results due to a different sleep environment.

Furthermore, we can not rule out the hypothesis that the improvement on motor scores as rated by the UPDRS Part III may have contributed to reduce depressive symptoms regardless of the effect of the 5-HTP.

The number of included patients was low, and a larger study is definitely essential to confirm and extend the results, also with a higher 5-HTP dosage and longer observation times.

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