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Preclinical Solutions for Insight in Premotor Parkinson

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1. Introduction

Parkinson's disease is second only to Alzheimer's disease, among the main chronic and progressive neurodegenerative disorders. Although neurodegenerative disease may appear at any age, the risk increases with ageing. Therefore, the increasing prevalence of neurodegenerative diseases is an increasing concern for ageing western societies. The problem has been well-recognized and triggered research efforts to develop strategies to limit the progression of neurodegenerative diseases, such as Parkinson's disease.

The clinical features of Parkinson's disease were first described by the English surgeon James Parkinson in his "Essay on the shaking palsy" in 1817. Notably, it took another hundred years after Parkinson's publication before the first Parkinson's disease brain pathology was described. The great breakthrough in Parkinson's disease started in the 1950s by the Swedish scientist Carlsson (Fahn, 2008) who recognized dopamine as the neurotransmitter involved in the pathological process. This subsequently led to research into parkinsonian brains by the Austrian scientists Ehringer and Hornykiewicz. They demonstrated that the level of the neurotransmitter dopamine in parkinsonian brains was dramatically reduced (Ehringer & Hornykiewicz, 1960). This reduction was caused by the degeneration of dopamine neurons in the *substantia nigra*. Only one year later the first patients were treated with the dopamine replacement drug L-dihydroxy-phenylalanine (L-DOPA) (Birkmayer & Hornykiewicz, 1961). L-DOPA therapy for Parkinson's disease was designed and described by the English physician Cotzias (Fahn, 2008).

Nowadays, treatment is still heavily dependent on dopamine replacement therapy, which is primarily aimed at symptom control and can be associated with severe side effects. Furthermore, with this treatment approach there is no prevention or retardation of dopaminergic neuron degeneration. Therefore, it would be a better approach to focus on a medical intervention in the cell death processes to stop or slow down progression in order to increase the quality of life of these patients. This strategy has been well-recognized (Jankovic, 2005; Philippens et al., 2010; Tolosa et al., 2009) and research efforts to develop neuroprotective treatment strategies is the current focus of both clinicians and basic scientists. To achieve this strategy, early identification of individuals at risk and an early start of neuroprotective treatment to prevent the progressive loss of neurons are important. Once it is established that a person is at risk of developing Parkinson's disease, progressive loss of neurons must be prevented.

Generally, patients enter the clinic with motor-related problems regardless of the fact that they have been suffering from non-motor problems, such as olfactory dysfunction, mood changes and sleep problems for years before the actual diagnosis. Since there are no specific early diagnostic biological markers for Parkinson's disease, the clinical diagnosis is still entirely based on the presence of the characteristic motor features that start after the time when more than 50 % of the dopaminergic neurons have already been lost. At this stage neuroprotective strategies can only have a limited effect. Therefore, it is essential to establish markers to identify subjects at risk before motor manifestation. James Parkinson already described the difficulty of the awareness of this slow progressive neurodegenerative process in the early stages of the disease. He wrote: "*So slight and nearly imperceptible are the first inroads of this malady, and so extremely slow its progress, that it rarely happens, that the patient can form any recollection of the precise period of its commencement*". This formulates well the obstacles in the investigation of the early stages of Parkinson's disease and prompts us to find markers for the early stage of Parkinson's disease in humans and in animal models. In particular, animal models offer an opportunity to link low-level neurodegeneration to disease manifestation and a possibility to investigate strategies for intervention therapy. Despite all the research performed during the last five decades, neither the cause of sporadic Parkinson's disease, nor a preventive method and an acceptable symptom treatment have been found. We emphasize that a shift should be made from a diagnosis that is based on motor complications towards early recognition of premotor symptoms supported by the first indications of Parkinson's disease pathology. The ultimate goal will be treatment of individuals at risk with a neuroprotective therapy, thereby significantly prolonging their normal life style.

2. Parkinson's disease

Normal control of movement is a result of the complex interplay of various groups of nerve cells in the central nervous system. Neurons in the basal ganglia (*striatum, pallidum, subthalamic nucleus* and *substantia nigra*) are the key players in motor function and are responsible for the fine-tuning of movements. They are regarded as components of several largely segregated basal ganglia-thalamocortical circuits serving cognitive, oculomotor and motor functions (Joshua et al., 2009). Most important to the motor disorder, Parkinson's disease, are a group of neurons located in the *substantia nigra*, situated in the ventral midbrain. Neurons of the *substantia nigra* communicate with other neurons in the basal ganglia through dopamine neurotransmission. At the time of Parkinson's disease diagnosis, based on typical motor symptoms, patients have already lost at least 50% of the dopamine neurons in the *substantia nigra* (Jellinger, 2008). Together with the loss of these neurons, dopamine synthesis and dopamine release are drastically reduced. Owing to the disturbances in the striatal-thalamocortical circuit as a result of the decline of dopamine, the reinforcing influence of the motor circuitry upon cortically initiated movements is reduced (Alexander & Crutcher, 1990; Wichmann & DeLong, 2003). Indeed, in non-human primate models of Parkinson's disease, the reduction of *substantia nigra pars compacta* output leads to decreased facilitation of cortical motor areas and subsequent development of akinesia and bradykinesia (Wichmann & DeLong, 2003). Together with this neurodegeneration in the *substantia nigra*, intraneural protein inclusions are found in the *substantia nigra* and other brain regions (Braak et al., 2003). The marked neurodegeneration in the *substantia nigra* together with the occurrence of protein inclusions called Lewy bodies form the post-mortem confirmation of Parkinson's disease diagnosis.

2.1 Etiology of Parkinson's disease

Although Parkinson's disease has been studied for almost 200 years, the precise mechanisms leading to progressive cell death still need to be resolved; the actual cause and the mechanism(s) associated with the pathogenesis of Parkinson's disease are still unknown. However, it has been proposed that several factors including oxidative stress, excitotoxicity, mitochondrial dysfunction, environmental toxins, proteasome dysfunction, inflammatory aspects and genetic defects may contribute to the neurodegenerative process causing Parkinson's disease. Whatever the cause is, it is clear that Parkinson's disease is a multi-factorial disorder resulting from the combined effect of age, environmental factors, genetic susceptibility and complex genetic-environmental interactions (Fig. 1) (Chan et al., 1998; Le Couteur et al., 2002; Migliore & Coppede, 2009; Schapira, 2009). Many epidemiological studies support the role of pesticide exposure in Parkinson's disease. For example, rural living (Chen et al., 2009), drinking well-water (Gatto et al., 2009) and occupation-based exposure (Goldman et al., 2005) are potential risk factors that support the above possibility.

Independent of the actual cause, neurodegeneration in Parkinson's disease is based on an endogenous excitotoxicity, i.e. a Parkinson's disease patient basically destroys, and has destroyed, his or her own *substantia nigra* neurons by endogenously generated activity, combined with disturbed homeostatic control of the neurons. There is an obvious inbalance between energy supply and demand in neurons occurring during activation, but this may be the consequence of over-excitation. An alternative explanation is that the problem is related to neuronal homeostatic maintenance processes. Since Parkinson's disease is a slowly progressing disease, it is likely that the excitotoxic state is not always present, and only occasionally induces neuronal death. An excitotoxic state may be directly based on over-excitation, or due to suppression of neuronal maintenance processes; or (more likely) both. Alternatively, factors related to neuronal maintenance processes may be of great importance for understanding slow gradual neurodegeneration. In neurodegenerative disorders, already weakened neurons may not survive glutamate concentrations that would not normally be lethal. These weakening factors may represent susceptibility processes, which are perhaps more easily influenced and would require pharmacological interventions which are less invasive than those currently in use.

2.2 Molecular and cellular mechanisms of Parkinson's disease

Recently, at least eight defined genetic loci have been associated with autosomal dominant or recessive familial Parkinson's disease, wherein thus far five causative mutations have been identified (Nuytemans et al., 2010). Mutations in the following genes have been reported to cause familial Parkinson's disease: alpha-synuclein (SNCA), leucine-rich repeat kinase (LRRK2), parkin (PARK2), PINK1 (PARK6) and DJ-1 (PARK7). Although these familial forms of Parkinson's disease are rare compared with the frequency of sporadic cases, they are very important for understanding the molecular basis or cause of disease pathology. The cellular processes involved are thought to be related with age, environment, genes or a combination of these. They are: mitochondrial dysfunction, glutamate excitotoxicity, oxidative stress, inflammatory responses and proteasome dysfunction (Alexi et al., 2000; Betarbet et al., 2000; Dauer & Przedborski, 2003; Jenner, 2003a; Philippens et al, 2010). In this regard, neurons may die by necrosis, caused by changes in ion dynamics, cellular swelling resulting in the disintegration of the cell and its organelles and removal of cell debris by phagocytosis. Neurons can also go into apoptosis initiated by exogenous

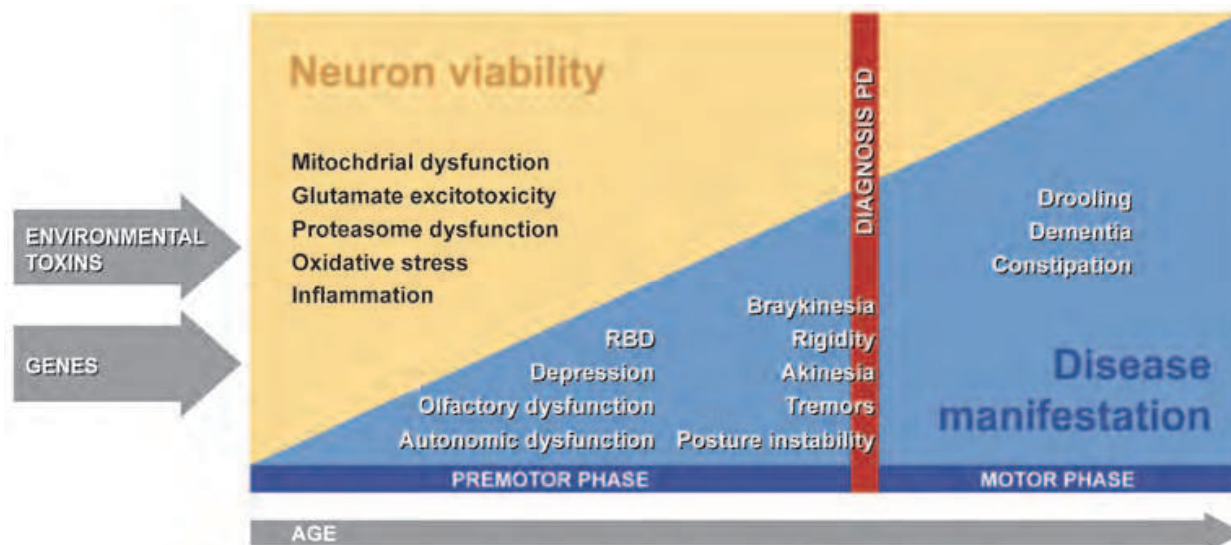toxins, which are mediated by e.g. oxidative stress and the release of cytochrome c by mitochondria.

Fig. 1. Schematic diagram depicting the pathology and symptomatology of Parkinson's disease.

The disease manifestation is divided into the premotor phase, before Parkinson's disease diagnosis and the motor phase starting around the time of diagnosis. The multi-factorial nature of the disease is depicted by the possible causes of Parkinson's disease: environmental toxins, genes and age that are depicted by the grey arrows. Neuron viability is reduced over time as shown in the yellow triangle with the proposed features that cause the degeneration of neurons in random order. The occurrence of symptoms increases over time as shown in the blue triangle with the different symptoms that occur at each phase of the disease. PD: Parkinson's disease; RBD: rapid eye movement sleep behavior disorder.

2.3 Parkinson's disease manifestation

Parkinson's disease is strongly progressive, meaning that the symptoms worsen with time. Even under currently available medication, motor incapacitation appears five to ten years after onset of the disease. The progressive neurodegeneration in Parkinson's disease results in a wide range of disabling motor and non-motor symptoms (Fig. 1). Generally, patients enter the clinic with motor-related problems when they are in their fifties. At the time of diagnosis they are suffering from bradykinesia (slowness of movements) and one or more of the other classic motor-related problems: tremor at rest (typically in the hands), rigidity of movements, akinesia (impaired movements) and/or postural instability (balance problems). Besides motor problems, patients may suffer from disturbing non-motor problems like depression, constipation and dementia. Since there are no early diagnostic biological markers for Parkinson's disease, clinical diagnosis is currently entirely based on the presence of the characteristic motor features. However, diagnostics based on live imaging of the dopamine transporter in brain scans and non-motor symptoms like olfactory dysfunction are under current investigation (Deeb et al., 2010). Many patients report having suffered from abnormal olfaction, mood disorders, autonomic dysfunction and sleep

problems years before the motor symptoms-based diagnosis (Berg, 2008; Tolosa et al., 2009). Of these non-motor symptoms, sleep problems are reported in around 80% of all Parkinson's disease patients (Tandberg et al., 1998; Garcia-Borreguero et al., 2003; Oerlemans & de Weerd, 2002). Sleep problems can range from reduced sleep efficiency, difficulty in turning in bed, to motor problems during rapid eye movement (REM) sleep, diagnosed as REM Sleep Behavior Disorder (RBD). The latter observation has been proposed as a useful preclinical biomarker for Parkinson's disease (Iranzo et al., 2006; Postuma et al., 2006). Together with the classic motor symptoms, these sleep problems are very disturbing for patients and their (bed) partners. All together, with or without medication, patients are heavily disabled by a wide range of problems, which invariably increase over time.

2.4 Neuroprotective treatment

Recently, deep brain stimulation (DBS) has gained popularity as a treatment for tremors in advanced Parkinson's disease (Sydow, 2008) by suppressing the neuronal firing pattern in the target area (*subtalamic nucleus*) either directly or by inducing the release of inhibitory transmitters (Hilker et al., 2008). Thousands of patients worldwide have undergone DBS treatment. Although temporarily effective, these Parkinson's disease therapies do not stop or reduce the neurodegenerative state and therefore do not actually cure the disease. The current priority in Parkinson's disease research is, therefore, to move beyond symptom control and to develop neuroprotective treatments.

Several strategies have been proposed which can protect the brain from neurodegeneration. One of these strategies is the neurorestorative cell therapy treatment, which is still under investigation. However, major ethical and practical issues need to be resolved before they can be tested in the clinic (Xi & Zhang, 2008). Therefore, we still rely on pharmacological approaches. Most of the neuroprotective compounds either act as anti-oxidants or as anti-apoptotic agents. Some of the anti-oxidants have already been tested in the clinic such as tocopherol, the monoamine oxidase B (MAO-B) inhibitor l-deprenyl, the mitochondrial stabilizer coenzyme Q10. The anti-apoptotic compound rasagiline has also been investigated (ParkinsonStudyGroup, 1993; Shults et al., 2002). Anti-apoptotic compounds such as neuro-immunophilin, pramipexole and ropinirole have also been tested for their neuroprotective efficacy in Parkinson's disease patients (Gold & Nutt, 2002; ParkinsonStudyGroup, 2002; Sethi et al., 1998).

Besides these drugs, some treatments are directed against inflammation, glutamate release or excitotoxicity, or addressing the disturbed mitochondrial energy supply or neuronal maintenance, thereby ultimately aiming at reducing apoptosis and necrosis of the dopamine neuron. Examples are riluzole, a versatile anti-excitotoxic compound and a possible candidate for neuroprotection in Parkinson's disease (Bensimon et al., 2009), and trophic factors like glial cell-derived neurotrophic factor (GDNF) (Nutt et al., 2003) and the dopamine replacement L-DOPA (Fahn, 2005). Some of the above mentioned compounds have shown promising neuroprotective effects in the clinic, whereas others have not fared well. The neuroprotective efficacy is generally measured by the delay in time before starting L-DOPA therapy, changes in Parkinson's disease symptoms, or imaging of dopamine markers. The following factors may lead to difficulties in assessing the effectiveness of a neuroprotective compound (Olanow et al., 2008; Ravina et al., 2003):

1. None of the outcome measures used in clinical trials directly reflect neurodegeneration;
2. The outcome measures were confounded with the symptomatic or pharmacological effects of the intervention;
3. Dosing to achieve neuroprotective action of a compound is often a guess, based on parameters which have been identified in animal studies, but may not be relevant in humans;
4. Diagnosis can be mistaken for other related parkinsonian disorders;
5. And patients included in the trials have already been diagnosed with Parkinson's disease and are thus in a progressive state of neurodegeneration.

Especially the fifth factor may be an important reason for the lack of neuroprotective effect, because the neurodegenerative process has already proceeded substantially before the first motor symptoms allowed the clinical diagnosis of Parkinson's disease in these patients.

Although several neuroprotective compounds are good candidates and have been tested in both animal models and patients, none have led to a neuroprotective treatment for Parkinson's disease approved by the Food and Drug Administration (FDA). Neuroprotection in patients remains the ultimate goal. However, it has to be combined with extensive preclinical screening, early diagnosis and exclusive neuroprotection markers.

3. Modeling Parkinson's disease

Studying Parkinson's disease neurobiology in combination with disease manifestation in humans is limited to clinical trials and post-mortem material. Therefore, in order to find new targets for neuroprotective therapies, the availability of adequate animal models would be an excellent asset in current Parkinson's disease research. Cell cultures or invertebrate models are useful (Botella et al., 2009; Schule et al., 2009) but they can only model Parkinson's disease to a certain extent.

Animal models should ideally mimic the main features of the disease pathology and additionally show the typical parkinsonian syndrome. In this regard, four scientific criteria have been proposed (van der Staay et al., 2009) to judge the validity of a model: face, predictive, construct and external validity. The dopamine deficiency observed in Parkinson's disease is the main cause underlying the pathophysiology of the motor symptomatology.

Animal models can feature the typical –preferably progressive- loss of dopamine neurons in the *substantia nigra* in combination with the associated dopamine reduction in the *striatum.* This is called face validity or the degree of descriptive similarity between the symptoms in the animal model and in humans affected by Parkinson's disease. Often the presence of the typical Parkinson's disease behaviors or the Parkinson's disease specific Lewy body formation can be an important addition to answer the research question addressed in an animal model. In the pharmacological context, predictive validity refers to the ability of a model to correctly identify the efficacy of a therapeutic strategy. Therefore L-DOPA-induced improvement of motor behavior is a key issue in animal models for Parkinson's disease.

Because of the multi-factorial nature of Parkinson's disease, construct validity is the most difficult scientific criterion in modeling idiopathic Parkinson's disease. Construct validity is the degree of similarity between the mechanisms underlying behavior in the model and those in the condition being modeled. Animal models can mimic the pathology and the symptomatology of the disease but not easily the etiology. However, factors like genes, environment and age can be altered separately or in combination in animal models thereby

ensuring construct validity to a certain extent. External validity represents the way results obtained using a particular model can be generalized or applied to and across populations. The ultimate Parkinson's disease model has not yet been described. However, there are several experimental models that meet the above criteria. Some Parkinson's disease models rely on selective neurotoxins to chemically destroy dopamine neurons or on precise targeting of the specific brain regions using stereotactic surgery. Others are focused on genetic defects.

Having a relatively short lifespan, mice are of interest for disease models owing to their rapid progression through the disease stages and their consistent neurological defect. Mutant mice are valuable models for investigating various pathological conditions that modify brain function either during development or in adulthood. There are also several pharmacologically induced Parkinson's disease models available, such as the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model (Schmidt & Ferger, 2001). However, unlike humans and monkeys, mice need a relatively high systemic dose of MPTP to induce dopamine neurodegeneration (Jackson-Lewis et al., 1995) and they show a relatively restricted Parkinson's disease-like symptomatology. Therefore, face validity is not optimal (Luchtman et al., 2009). Furthermore, the behavioral changes due to Parkinson's disease induction recover very fast after the MPTP challenge (Schmidt & Ferger, 2001). Thus, mice are mainly of interest for neuropathology and molecular changes after neurodegeneration of dopamine neurons and not suited for extensive research into clinically based symptomatology.

While, physiological and pharmacological questions can often be studied well in rodent models, issues concerning complex behavior can be addressed more accurately in primates. Therefore, non-human primate models are preferred in clinically focused behavioral studies because their disease manifestation can be translated directly to the human situation (Annett et al., 1994; Di Monte et al., 2000; Eslamboli, 2005).

In this regard, the common marmoset (*Callithrix jacchus*) is an established model in neuroscience. Compared to humans, these monkeys have a similar *striatum* structure, hand-foot use and motor behavioral reaction (construct validity), and for example a similar response to dopamine replacement therapy (predictive validity) (Blanchet et al., 2004; Eslamboli, 2005; Hardman et al., 2002; Jenner, 2003b). Additionally, non-human primates are genetically closer to humans than rodents and react similarly to pharmacological interventions (face validity) (Smith et al., 2001). The small and easy to handle marmoset is thus of major importance as a model in behavioral studies (Willner, 1986).

Non-human primates are generally appreciated as model species because of their great validity for simulating conditions in humans. Monkeys are, like humans, very sensitive to MPTP (Burns et al., 1983; Jenner et al., 1984) and after this treatment they express many features of clinical Parkinson's disease which might reflect their genetic, physiological and behavioral proximity to humans. They have a similar *striatum* structure, similar hand-foot use and comparable responsiveness to all dopaminergic medications known to be effective in Parkinson's disease (Eslamboli, 2005); this makes them a valuable addition to the range of available Parkinson's disease models. They also have optimal face validity and predictive validity. Old-world monkeys with high cognitive abilities, such as macaque monkeys (Burns et al., 1983) and baboons (Hantraye et al., 1993) are interesting because they can handle complex behavioral tasks enabling testing of cognitive deficits in the late stage of Parkinsonism. New-world monkeys, such as squirrel monkeys, capuchin monkeys and common marmoset monkeys are, although somewhat less sensitive to MPTP, especially

useful because of the aforementioned abilities, but also for their size and are consequently easy to handle in the laboratory. The popular marmoset MPTP model offers several advantages in studying disease therapies and neuroprotective methods (Philippens, 2009). Unlike rodent models (Jackson-Lewis et al., 1995; Mandel et al., 2003; Meredith et al., 2008), MPTP-treated marmoset monkeys show optimal face validity with a wide range of parkinsonian behaviors (Jenner et al 1984; van Vliet et al., 2006) including the L-DOPA-induced dyskinesia (Visanji et al., 2006) and hallucinations (Fox et al., 2006). The ''clinical'' condition of the parkinsonian state in this MPTP monkey model is generally focused on the motor symptoms. Motor symptoms reported in marmosets use extensive rating scales (Iravani et al., 2003; Jenner, 2003b; Pearce et al., 1996; van Vliet et al., 2006) and tests towards locomotor activity (Obinu et al., 2002; van Vliet et al., 2006), hand-eye coordination (Annett et al., 1994; van Vliet et al., 2006) and akinesia and jumping behavior (Verhave et al., 2009). Non-motor symptoms are apathy (van Vliet et al., 2006) and bladder problems (Albanese et al., 1988). More recently, we can also add sleep related symptoms to this list (Verhave et al., 2011). Because of the striking similarity in sleep macrostructure between marmoset monkeys and humans, changes in sleep due to MPTP treatment can be used as a premotor sign in early stage of Parkinson's disease. A more in-depth insight of the non-motor symptoms would be a valuable addition to this model in order to investigate the effect of dopaminergic drugs as reviewed by Jenner (Jenner, 2009).

3.1 Chemically induced Parkinson's disease

The most potent toxin to induce Parkinson-like dopamine neurodegeneration is 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP is a neurotoxin that easily crosses the blood-brain barrier and is specific for dopamine neurons. The MPTP model induces face validity through a specific lesion in the *substantia nigra* and it shows predictive validity with the use of L-DOPA. The MPTP model is actually environmentally induced parkinsonism, and therefore has construct validity to a certain extent. Since MPTP induces reproducible Parkinson's disease in mice, monkeys and humans it offers appropriate external validity.

Other useful toxins are the non-specific 6-hydroxidopamine (6-OHDA), rotenone and paraquat. 6-OHDA is recognized by *substantia nigra* neurons as dopamine and is taken up by the cell where it then exerts its toxic properties. As 6-OHDA does not cross the blood-brain barrier it needs to be locally administered, which is not a trivial procedure. As a result of this local administration, the severity of the lesion depends on the separation of the point of application from the region of interest, which makes this model less suitable for studying the molecular mechanisms of neurodegeneration (Bove et al., 2005). An advantage of this model is that unilateral 6-OHDA lesions, generally used in rats, have proven to be very reproducible over time, in which the non-infused hemisphere can serve as intra-animal control (Blandini et al., 2007). Unlike paraquat which offers contradictory results in mice, repeated systemic administration of rotenone, which easily penetrates the blood-brain barrier, is another potential alternative for inducing Parkinson's disease in experimental animals (Sherer et al., 2003; Schmidt & Alam, 2006). Both paraquat and rotenone affect neurons by disturbing processes such as the glutamate balance, increasing reactive oxygen species (ROS) production, the mitochondrial respiration or misfolding of proteins as reviewed by Bove et al. 2005.

3.1.1 MPTP

Since the serendipitous discovery of the neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in young Calnifornian drug users in the 1980s (Langston et al.,

1983), it has become the preferred agent to induce parkinsonism in laboratory animals. This drug represents the most important and most frequently used neurotoxin in animal models. MPTP selectively damages dopaminergic neurons (Javitch et al., 1985), which invariably leads to impaired dopamine neurotransmission.

After entering the brain, glia cells facilitate the conversion of MPTP into 1-methyl-2,3-dihydropyridinium (MPDP$^+$) by the enzyme MAO-B. Thereafter, the actual toxic metabolite, 1-methyl-4-phenylpyridinium (MPP$^+$), is formed by oxidation. MPP$^+$ leaves the glia cells and enters the dopamine cells via the dopamine transporter. Once inside the neuron MPP$^+$ is taken up in vesicles by the vesicular monoamine transporter and then into mitochondria using an energy demanding process (Del Zompo et al., 1993). In mitochondria, MPP$^+$ blocks the electron transport enzyme ubiquinone oxidoreductase (complex I) (Nicklas et al., 1985) and leads to a reduction in cellular ATP.

4. Markers for the early phase of Parkinson's disease

A maximally beneficial effect of neuroprotective therapies in the treatment of Parkinson's disease can only be achieved with early diagnosis and an early intervention. But unfortunately, early diagnosis of Parkinson's disease may be difficult. Nowadays the clinical diagnosis of Parkinson's disease is still primarily based on overt clinical motor symptoms, such as unilateral rest tremor, reduced arm swing, and slowed hand movement. These symptoms emerge relatively late in the course of the underlying neurodegenerative process when more than 50 % of the dopamine neurons are already lost.

Therefore, the identification and validation of biomarkers, such as premotor symptoms, are critical to facilitate the early diagnosis of Parkinson's disease. Examples of premotor symptoms of early Parkison's disease are olfactory decline, alterations in mood and autonomic function, and most notably disturbed sleep (Berg, 2008; Tolosa et al., 2009). For instance, complaints of insomnia are reported in approximately 80% of all Parkinson's disease patients (Oerlemans & de Weerd, 2002; Tandberg et al., 1998), and excessive movement during sleep frequently occur in Parkinson's disease patients (van Hilten et al., 1994).

But the slow onset of the clinical impairments in relation to the progression of neurodegeneration makes studies correlating neuropathology to symptom manifestation in human subjects difficult. Therefore, research efforts should be focused on the identification of biomarkers for early diagnosis and neuroprotective treatment in relevant animal models of the disease with an emphasis on human validity. The non-motor symptoms of Parkinson's disease have only recently started to become of interest to the scientific community (Park & Stacy, 2009). In animal models this is a very new field so that non-motor parameters are generally not yet part of the symptom description. However, there are reports on olfaction problems in the MPTP-treated marmoset (Miwa et al., 2004), and several studies on sleep problems in non-human primates (Almirall et al., 1999; Barraud et al., 2009), as well as on constipation (Anderson et al., 2007).

Hence, the MPTP treated marmoset monkey may fill this gap and provide insights into the course of non-motor effects in relation to the underlying neurodegeneration of the brain. Since the focus in Parkinson's disease research has recently shifted from the motor phase to the premotor phase of the disease (Berg, 2008; Marek & Jennings, 2009; Philippens et al., 2010; Stephenson et al., 2009; Tolosa et al., 2009; Tolosa & Poewe, 2009), it is of particular interest to investigate further the validity of the classic MPTP model of Parkinson's disease for the study of premotor symptoms. The stages in which premotor symptoms are apparent

and precede the motor phase of Parkinson's disease need to be identified, in order to investigate early pathological processes of the disease and to develop early treatment approaches. In addition, as there are currently still no blood or cerebrospinal fluid biomarkers of Parkinson's disease, we rely completely on non-invasive markers based on the early premotor symptoms.

4.1 Brain imaging

Post-mortem pathological changes in the brain can be used as a definitive diagnostic marker for Parkinson's disease. However, for the living patient during the early stage of the disease this diagnostic tool is of no use in the clinic. For animal research post-mortem necropsy studies can be very useful for measuring the efficacy of a neuroprotective treatment at selected time points. MPTP induces selective lesions in the dopaminergic neurons of the *substantia nigra pars compacta*. The damage to the dopaminergic neurons can be identified with tyrosine hydroxylase (TH) staining, which is the first and rate-limiting enzyme in the synthesis of the catecholamines, and is often used as a quick and sensitive method to visualize surviving dopaminergic neurons (Pearson et al., 1983; Waters et al., 1987). Beside the immunohistochemistry, neurochemical changes in the striatum, such as the level of dopamine and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), but also other monoamines, such as noradrenaline and serotonin, can be used to quantify the severity of neurodegeneration (Gerlach et al., 1991). Important in the interpretation of these data is the possible discrepancy between the TH-positive neurons and surviving dopamine neurons as it has been suggested that MPTP can reduce TH activity without cell loss (Tatton et al., 1990).

Nevertheless, these neuropathological measures are invasive and static read-out values. Dynamic changes in the brain due to cell death, recovery and compensation mechanisms cannot be explored with these techniques. Brain imaging techniques, such as positron emission tomography (PET) using radioactive [^{18}F]6-fluoro-DOPA (F-DOPA), single proton emission computed tomography (SPECT) using the dopamine uptake ligand [^{123}I]beta-CIT, and magnetic resonance imaging (MRI) can cover this lacuna as these techniques are non-invasive and can be repeated over time. The PET scan quantifies the reduction of dopamine metabolism caused by dopaminergic neuronal death. The SPECT scan targets the dopamine transporter on the dopamine neuronal terminals indicating the loss of these axons. Dopamine transporter imaging using SPECT (DatSPECT), a technique that has been approved for use in Europe, offers one way of improving diagnosis of Parkinson's disease. There is a high density of dopamine transporter target sites in the striatum, the region of the brain that is primarily affected by Parkinson's disease. Continued neuronal degeneration with Parkinson's disease progression shows up as diminished uptake of the radiotracer on the dopamine transporter imaging. In clinical studies, MRI and magnetic resonance spectroscopy (MRS) are very versatile techniques that examine structural and physiological processes in living organism and are widely used in clinical and experimental research (Dijkhuizen & Nicolay, 2003). These neuronal imaging techniques may be useful for the early detection of Parkinson's disease. Therefore, investigational imaging techniques could soon provide earlier and more definitive diagnosis of Parkinson's disease.

In animal studies, these techniques can also have scientific and ethical benefit as the animal acts as its own control thereby reducing animal usage for research. The advantage is that these non-invasive techniques can be applied repeatedly over a prolonged period with the opportunity of following the progress of the disease over time.

4.1.1 Magnetic resonance imaging and spectroscopy (MRI and MRS)

A typical **MRI** technique used for Parkinson's disease research is T2-weighted imaging in which changes in signal intensities are partly due to an altered water content of a tissue, mostly caused by the presence of extracellular edema (Dijkhuizen & Nicolay, 2003). Earlier examinations with T2-weighted imaging on the effect of MPTP intoxication in animals showed changes in relevant brain areas like the *substantia nigra pars compacta*, *caudate nucleus* and *putamen* (Miletich et al., 1994; Podell et al., 2003; Zhang et al., 1999).

MRS, on the other hand, visualizes signals from carbon-bound protons from various metabolites (Kauppinen & Williams, 1994). But with MRS only few metabolites can be examined. The most frequently used neuronal marker with MRS is N-acetyl aspartate (NAA) (Gujar et al., 2005; Castillo et al., 1996). Reductions of NAA levels have been observed in the striatum and *substantia pars compacta* in parkinsonian mice, cats, cynomolgus monkeys and marmoset monkeys (Boska et al., 2005; Brownell et al., 1998; Podell et al., 2003; van Vliet et al., 2008). An example of brain imaging of a marmoset brain slice from a region of interest for MRS analysis is given in figure 2.
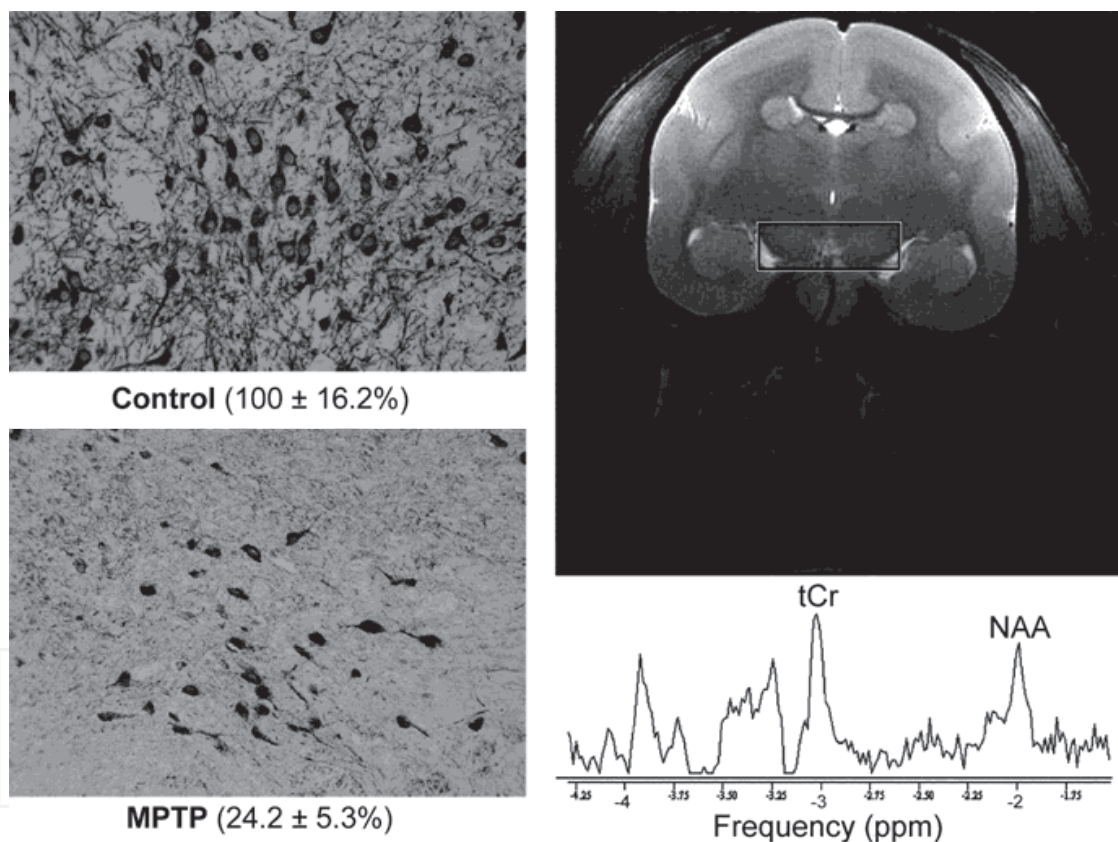

Fig. 2. Examples of neuronal brain imagings of marmoset monkey brain.

Left: An example of post-mortem immunochemistry imaging TH staining of neurons in the *substantia nigra pars compacta* in a healthy contol marmoset monkey (100 % TH-positive neurons) and of a monkey treated with a total dose of 6 mg/kg MPTP (24 % TH-positive neurons). Right side: A magnitic resonance brain image of a marmoset brain slice (Bregma -0.6 mm) with an outlined region for single voxel spectroscopy (squared box) in which the *substantia nigra* is located for the MRS analyses. Below is an example of an original MRS spectrum, which is used for data analyses of proton emission. NAA: N-acetyl aspartate; tCr: total (phospho) creatine.

In the MPTP treated marmoset monkey Parkinson's disease model magnetic resonance techniques were validated as a supplement for Parkinson's research towards neuroprotection (van Vliet et al., 2008). Both MRI and MRS were applied to investigate the neuroprotective effects of the vigilance-stimulating compound modafinil (Modiodal®) using a 4.7 T NMR spectrometer (Varian, Palo Alto, California). Modafinil was chosen because of its neuroprotective effects on dopamine neurons in the *substantia nigra pars compacta* in the marmoset MPTP model (Jenner et al., 2000; van Vliet et al., 2006).

In this particular study the MPTP intoxication resulted in a significant reduction of about 75 % of TH positive dopaminergic neurons as shown in figure 2, whereas this neurodegeneration level was significantly reduced to only 39 % in the modafinil treated parkinsonian monkeys.

In the MRI scan, MPTP intoxication resulted in a tendency for reduced T2 relaxation times in the *substantia nigra*: we found a tendency to decreased relative relaxation times in the placebo treated parkinsonian monkeys, which was not observed following modafinil treatment (van Vliet et al., 2008). This was in contrast with earlier reports in which an increase of T2-weighted signal intensities was described in the *substantia nigra* and dopaminergic projection areas (Miletich et al., 1994; Podell et al., 2003; Zhang et al., 1999). The differences between these and our studies were the extended time between MPTP treatment and the execution of the brain-imaging scan in our study compared to the other studies, in which the interval was several hours to several days. Also, we used a lower dose of MPTP in our study, which was only 6 mg/kg. This approach prevented extracellular edema, which generally results in an increase in T2 relaxation time (Dijkhuizen & Nicolay, 2003). Indeed, in contrast to the other studies, we found a similar reduction of the T2 relaxation time in the *substantia nigra* as has also been reported in Parkinson's disease patients (Kosta et al., 2006).

In the MRS scan MPTP intoxication resulted in a reduction of the NAA/total creatine (tCr) ratio: we found a significant reduction of the NAA/tCr ratio in the placebo treated parkinsonian monkeys. On the other hand, the NAA/tCR ratio after modafinil treatment was significantly increased following MPTP-intoxication compared to baseline values (van Vliet et al., 2008). The decrease in the NAA/tCr ratios has also been seen in other MPTP studies. Mice also show a clear decrease in the absolute NAA concentration in the *substantia nigra* both at 2 and 6 days after MPTP intoxication (Boska et al., 2005). Cats show decreased NAA/tCr ratios in the striatum 12 hours after MPTP intoxication (Podell et al., 2003). Furthermore, chronic MPTP intoxicated cynomolgus monkeys show a persistent reduction in NAA/tCr ratio in the caudate and putamen (Brownell et al., 1998).

The neuroprotective action of modafinil, measured in the brain by immunohistochemistry TH staining and brain imaging using MRI and MRS shows a clear correlation with the observed clinical parkinsonian symptoms, which indicates the value of both markers in neuroprotection research (van Vliet et al., 2008). It can be concluded that MRS (NAA/tCR ratio) is a valuable tool for neuroprotective research in the MPTP intoxicated marmoset as correlations indicate a clear relationship between motor functional deficits and measurements of brain damage.

4.2 Sleep

Unlike the nocturnal preference and fragmented pattern of sleep in mice and rats, the architecture of marmosets' sleep resembles that of humans (Philippens et al., 2004; Verhave et al., 2011). Marmosets are diurnal and, as in humans, their night sleep architecture consists

of a recurring pattern of cycles with light, deep and REM sleep. Further, quantifying the different stages in marmoset monkeys can be performed with the classical sleep scoring system directly adapted from human scoring (Rechtschaffen & Kales, 1968). Because of the striking similarity in sleep macrostructure between marmoset monkeys and humans, a demonstration of early abnormalities during sleep in the marmoset MPTP model would be of significant value as potential biomarkers for the early stage of idiopathic Parkinson's disease.

Although rodent studies are frequently used for Parkinson's disease research, the nocturnal nature of these animals' behavior and the short sleep bouts (Monaca et al., 2004; Yi et al., 2007) make them less suitable as models for sleep in Parkinson's disease. Nevertheless, sleep changes have been reported in the rat (Lima et al., 2007). In rats, MPTP induces a temporary reduction of REM sleep and increases sleep efficiency (Lima et al., 2007). However, possible changes in muscle tone during REM sleep were not addressed in this study. In the marmoset MPTP model, with a mild parkinsonian state, resembling an early phase of Parkinson's disease in humans, selective abnormalities in muscle tone during REM sleep phases were found (Verhave et al., 2011). In figure 3 an example is shown of electroencephalogram (EEG) traces during REM sleep combined with the electromyogram (EMG) indicating complete atonia during the normal healthy situation and with severe muscle tone indicating the presence of RBD.
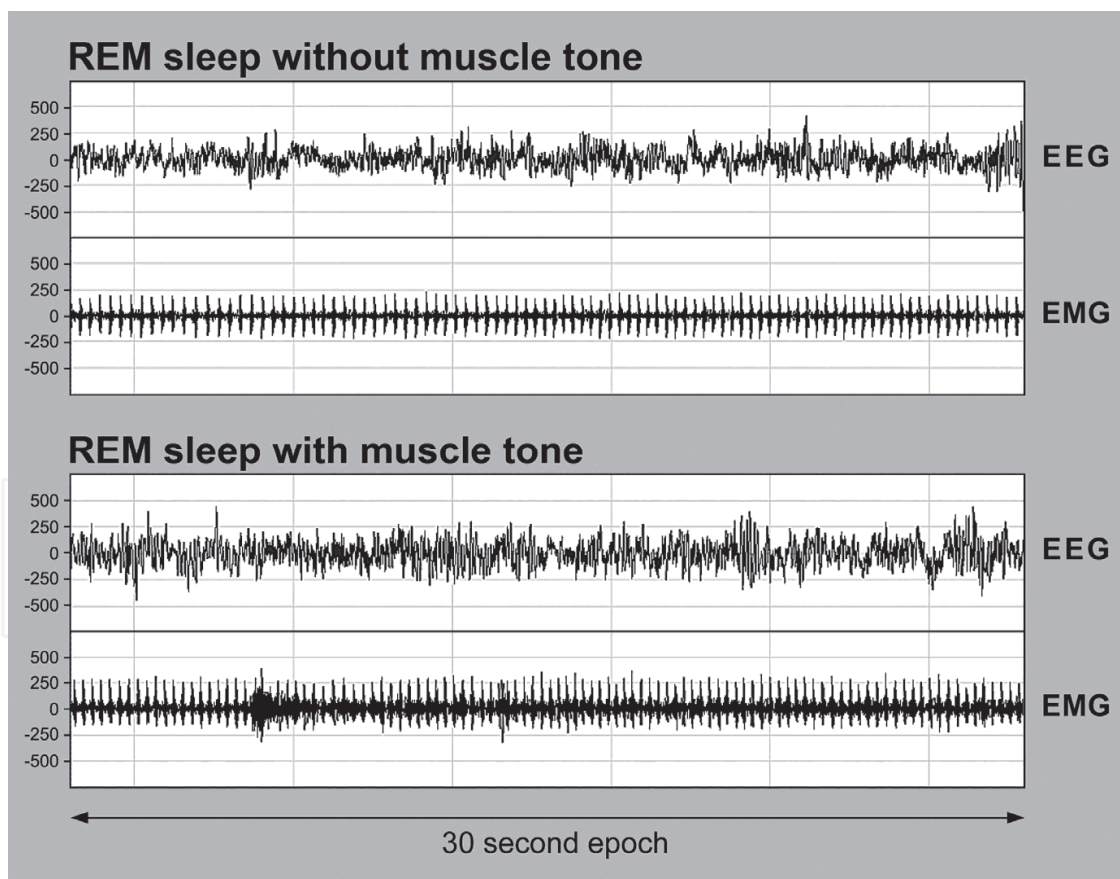

Fig. 3. Example of EEG and EMG epoch during REM sleep with and without RBD.

A 30-second epoch of an electroencephalogram (EEG) for measuring the sleep stage and electromyogram (EMG) for measuring the muscle tone during REM sleep of a normal

healthy marmoset monkey (upper level): the 30-second trace consists of random fast EEG while the EMG showed no muscle activity, and of a marmoset monkey suffering from RBD (bottom level): the trace consists of random fast EEG in the presence of 50-100% muscle tension in the EMG (Verhave et al., 2011).

The regular spikes are artefacts of the heart rate.

4.2.1 Rapid eye movement behavior disorder as a sleep marker for early Parkinson's disease

Rapid eye movement sleep behavior disorder (RBD) is characterized by increased muscle activity during rapid eye movement (REM) sleep, which can lead to injury either to oneself or to a bed partner. The core symptom of RBD, namely the lack of normal muscle atonia during REM sleep (Comella et al., 1993; Ondo et al., 2001), can emerge in two ways: (1) tonic muscle activity characterized by at least 50% of the time muscle activity in a 30-second REM sleep epoch or (2) phasic muscle activity and twitches within 30-second epochs (Lapierre & Montplaisir, 1992). RBD is a disorder of considerable interest for understanding early pathological processes in Parkinson's disease. At least one-third of Parkinson's disease patients have increased and irregular chin muscle tone during REM sleep (Comella et al., 1993; Gagnon et al., 2002) and many meet the criteria for RBD. More importantly, disturbances in sleep usually begin years before Parkinson's disease is diagnosed (Iranzo et al., 2005; Postuma et al., 2006; Tolosa et al., 2009). RBD is a key symptom during the early phases of Parkinson's disease, since one-third of all patients initially diagnosed with RBD are later diagnosed with Parkinson's disease within 3 to 13 years after the initial RBD diagnosis (Gagnon et al., 2002; Iranzo et al., 2006; Schenck et al., 1996). Moreover, 40% of all RBD cases reported eventually go on to develop a neurological disorder, most notably Parkinson's disease (Ferini-Strambi & Zucconi, 2000).

We investigated the effects of MPTP treatment on sleep architecture in marmoset monkeys, with special attention to RBD-like changes in muscle tone during REM sleep (Verhave et al., 2011). Because of the direct link between RBD and Parkinson's disease (Gagnon et al., 2002; Iranzo et al., 2005; Iranzo et al., 2006; Postuma et al., 2006; Schenck et al., 1996; Stiasny-Kolster et al., 2005), we evaluated different sleep components, particularly the REM sleep chin muscle disturbances in the marmoset MPTP model. To achieve this, the general sleep characteristics and the muscle tone (EMG) changes during REM sleep in the marmoset MPTP model of Parkinson's disease were investigated.

MPTP-treated marmosets showed no reduction in total sleep time, time spent in REM sleep, light or deep sleep and wake time after sleep onset (Verhave et al., 2011). However, MPTP significantly increased endogenous muscle tone during REM sleep ($p < 0.05$) (Fig. 3). We also determined the distribution of muscle tone as a percentage of the total time in REM sleep (Fig. 4). REM epochs were categorized according to muscle tone as either being absent or present in one of three predefined levels. Epochs with muscle tone more than 50% of the time were found to be rare in the control monkeys. However, they were significantly more frequently scored in animals treated with MPTP: MPTP, but not saline, increased the occurrence of epochs with muscle tone more than 10% of the time (Fig. 4: 32.8 ± 4.5 vs. 22.4 ± 2.9 % of REM epochs; $p < 0.05$). There were also significantly less epochs without atonia during the nights after MPTP treatment compared to the baseline values (Fig. 4: 38.3 ± 4.1 vs 66.3 ± 9.1 % of REM epochs; $p < 0.05$). Furthermore, discriminant analysis showed that after treatment, the number of epochs with 10% muscle tone during REM sleep classified 80% of the animals correctly and the epochs with 3 or more twitches added the remaining 20% of

this classification (Verhave et al., 2011). These two variables together classified the MPTP-treated monkeys up to a maximum of 100% (p < 0.05).

As the motor behavior in these MPTP-treated monkeys was disturbed to a mild extent, the Parkinson's disease induction could be described as a model of mildly Parkinsonian signs.
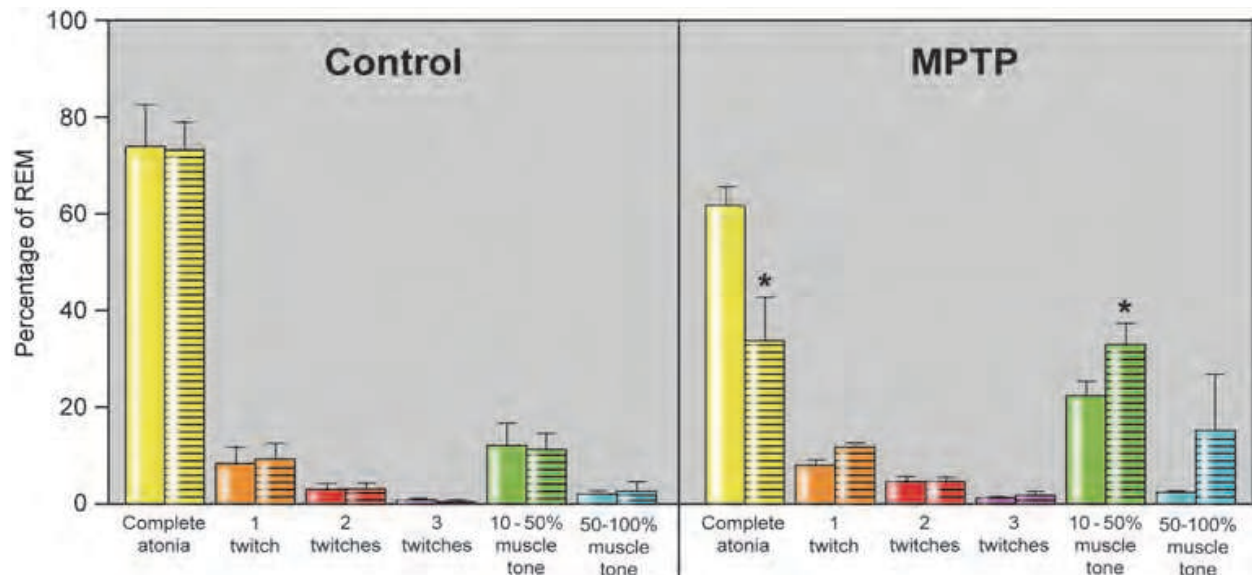

Fig. 4. Muscle tone during REM sleep in the marmoset monkey.
REM sleep epochs with tone as a percentage of REM sleep in the placebo control group (n=4) and the MPTP-treated group (n=5) before (baseline, solid bars) and after saline control or MPTP treatment (striped bars). Asterisks indicate significant differences between baseline value and after treatment (p < 0.05 Friedman) (Verhave et al., 2011).

In the paper of Verhave et al. (2011) it was explained that the MPTP induced changes in REM sleep muscle tone are presumably due to changes in dopamine neurotransmission. Reduction in dopamine in the *substantia nigra* caused by MPTP exposure results in a decrease dopamine signalling to its receptors localized within the *striatum* (Levey et al., 1993). Clinical conditions affecting dopamine, such as those seen in Parkinson's disease, also alter sleep architecture (Comella et al., 1993), which result in changes in REM sleep (Dahan et al., 2007) and muscle tone during REM sleep (Fantini et al., 2003; Garcia-Borreguero et al., 2002). Therefore, nigrostriatal neurons whose axons are located in the *striatum* are assumed to play a major role in the regulation of REM sleep. On the other hand, it has been suggested by Verhave et al. (2011) that the degenerative process in Parkinson's disease is initiated in the medulla, advances to the pons, and subsequently targets the midbrain (Braak et al., 2003). Thus, the presence of RBD might also reflect early involvement of non-dopamine medullary and/or pontine REM sleep-related structures (Iranzo et al., 2005). Therefore, it is suggested that these structures, which are closely connected to the *substantia nigra* pathways are affected by an imbalance of dopamine levels (Lai & Siegel, 1990) which would precede the actual neurodegenerative process. This is an important indication for the early onset of RBD during the neurodegenerative process leading to Parkinson's disease.

In conclusion, the MPTP-treated marmoset provides a new opportunity for quantitative studies on the mechanisms and intervention strategies of RBD and the premotor phase of Parkinson's disease.

5. Implications for the clinic

The current standard for diagnosing patients with Parkinson's disease remains the professional opinion of a neurologist based on a thorough neurological assessment. Earlier diagnosis of Parkinson's disease is becoming more and more important because of an ongoing shift in treatment, away from symptom control drugs and toward neuroprotective drugs that interfere with the cell death processes in order to stop or slow down the progressive pathophysiologic alterations in the brain. This is crucial because treatment should start at a very early phase of the disease to save as many neurons as possible. As the current treatment regimes don't stop or slow down the neurodegenerative process, research efforts are changing from symptom control strategies towards neuroprotection. From this point of view research models should be adapted to these new questions that ask for biomarkers for the early premotor stage of the disease and diagnostic tools to spot patients or individuals at risk and should, therefore, be based on early premotor indicators.

5.1 Neuroimaging

Neuroimaging can be used as an important tool for early diagnosis as well as for preclinical research into the early stages of the neurodegenerative diseases. However, it is not clear at what stage neuronal cell loss becomes evident in PET and SPECT imaging. At the onset of the disease manifestation, when more than 50% of the dopaminergic neurons in the *substantia nigra* are already lost, there is only a 30% reduction of putaminal F-DOPA measured by PET scan (Berg, 2006). As these techniques involve the exposure of subjects to radiation, they cannot be recommended for identifying individuals at risk for Parkinson's disease. An alternative could be MRI and MRS. In marmoset monkeys as well as in Parkinson's disease patients a reduction of the T2 relaxation time in the *substantia nigra* was found using MRI techniques (Kosta et al., 2006; van Vliet et al., 2008). The reduction of the T2 weighted signals, as was found in our MPTP-treated marmoset monkeys, is presumably caused by Parkinson's disease-related iron deposition. Iron creates magnetic field inhomogeneities that dephase nearby water protons resulting in a shortening of the T2 relaxation time (Kosta et al. 2006). Interestingly, iron deposition has been reported in the *substantia nigra* in MPTP models (Mochizuki et al., 1994; Temlett et al., 1994). Although the exact function of the increased iron levels is unknown, it is suggested that they contribute to oxidative stress in Parkinson's disease and MPTP models (Yantiri et al. 1999). Oxidative stress is known to be one of the features responsible for the initial triggering at the start of the neurodegenerative process (Philippens et al., 2010). In addition to the MRI measures, the NAA/tCR ratio, measured by MRS, is able to predict the disease state of the animal: a decrease in NAA/tCr ratio is associated with worsening of behavior after MPTP intoxication (van Vliet et al., 2008). This correlation suggests that NAA/tCr ratio measurement may be useful for studying the effects of neuroprotective drugs on the metabolic state of a neuron. A high correlation was found between NAA levels, measured with the MRS technique, and TH positive neurons, measured with the immunohistochemistry, in a mouse MPTP model (Boska et al., 2005) indicating that these parameters both predict neuronal damage.

5.2 Rapid eye movement behavior disorder

Another approach for detecting the early stage of Parkinson's disease is measuring premotor signs, such as the RBD, which is one of the most important indicators of developing

Parkinson's disease. The MPTP-treated marmoset model can be used for further studies into the mechanisms of RBD and sleep disturbances in the premotor symptom phase of Parkinson's disease, i.e., when patients can be diagnosed with RBD but not with Parkinson's disease (Gagnon et al., 2002; Iranzo et al., 2006; Schenck et al., 1996). However, the stringent criteria for RBD described in human studies (50-100% muscle tone per epoch) were not met by the parkinsonian marmosets in our study. The International Classification of Sleep Disorders (2005) describes RBD as the presence of REM sleep without atonia, and disruptive behavior during sleep. The atonia, normally observed during REM sleep, is interrupted by either short (phasic, 2-3 seconds) or long (tonic, 20-30 seconds) episodes of EMG activity (Gagnon et al., 2002; Iranzo et al., 2005; Lapierre & Montplaisir, 1992). Then again, a significant change in tonic activity is definitely apparent in our experimental animals, which suggests an RBD-like phenomenon. Indeed, an alternative and more suitable measure of RBD may be muscle activity as a percentage of REM sleep, given the variable outcome of polysomnogram measurements from 62 diagnosed patients (Mayer et al., 2008). This interpretation is supported by the parameters proposed by Mayer and colleagues (Mayer et al., 2008). For instance, the marmosets show a slight increase in phasic activity and a definite increase in tonic activity as a percentage of total REM sleep. The slight increase in phasic activity of EMG bursts or twitches (0.1-5 seconds) was seen in the epochs with one single twitch and the increase in tonic EMG was seen in the epochs with more than 10% tone of the time. This corresponds with EMG activity in at least one-third of the 30-second epochs.

5.3 Translational aspects

The translation of fundamental research into the clinic relies in the first place on appropriate animal models that show the pathological hallmarks and motor deficiencies of Parkinson's disease. It is of vital importance that these animal models actually mimic the clinical features of Parkinson's disease to the extent that the outcome is relevant. This can be determined by evaluating the marmoset MPTP model using scientifically-based criteria: face, predictive, construct and external validity as previously noted by Van der Staay and his colleagues (van der Staay et al., 2009).

Face validity: Based on the literature and our own findings, we can conclude that the marmoset model offers face validity in the sense that homologous neuro-anatomical structures are affected in this animal model and in Parkinson's disease as well (Jenner et al., 1984; Meredith et al., 2008). Changes induced in these structures result in neurodegeneration in the *substantia nigra* and reduced levels of dopamine in the *striatum*. The size of the lesion predicts the phenotype (van Vliet et al., 2008). Limited symptomatology in combination with the restricted damage of the dopamine neurons in the *substantia nigra* suggests that this model mimics an early Parkinson's disease patient (Tolosa et al., 2009).

Predictive validity: In the case of dopamine replacement therapy, predictive validity is certainly true for the marmoset model (Jenner, 2003b). However, for neuroprotection this model has not yet been very productive. Promising neuroprotective agents generated in the lab, have still not led to systematic treatment in patients (Olanow et al., 2008). This delay is mainly due to shortcomings of the models currently in use, the unknown cause of the disease and the difficulties for accurately estimating neuroprotection in the clinic.

Construct validity: Because the nature of the disorder is not well understood, construct validity is the most difficult scientific criterion to assess in modeling idiopathic Parkinson's disease. Animal models can only really mimic the pathology and the symptomatology of the disease but not its etiology. However, the use of MPTP with which neurodegeneration was

first discovered in humans, represents the best toxin available for studying Parkinson's disease as its derived symptoms are indistinguishable from those of idiopathic Parkinson's disease (Ballard et al., 1985). The MPTP-treated marmoset is superior in behavioral assessment, as it shows a complete range of behavioral characteristics associated with Parkinson's disease (Eslamboli, 2005; Jenner, 2009; van Vliet et al., 2006). Additionally, the report of non-motor symptoms such as sleep impairments and neuroimaging markers brings this model even closer to human Parkinson's disease (van Vliet et al., 2008; Verhave et al., 2011). Therefore, the striking similarities between human Parkinson's disease and MPTP-induced Parkinsonism in non-human primates strongly supports to construct validity in the MPTP models.

External validity: The non-human primate model is very popular in scientific research resulting in substantial reports about the degeneration of dopamine neurons in combination with motor functional loss (Meredith et al., 2008; Philippens et al., 2010; Speciale, 2002). In the end, it is the scientific question that should be a predominant factor in deciding of which specific animal model to choose. In regard to the early onset of the disease, the marmoset monkey is in favor of other animal models. Parkinsonian behavior of MPTP non-human primates is comparable to human Parkinson's disease symptoms (Przedborski et al., 2001). Clinical assessment of Parkinson's disease symptoms is mostly done with the UPDRS (unified Parkinson's disease rating scale). Rating scales have been adapted for MPTP non-human primates, for instance the 'clinical score', a rating scale that includes cardinal clinical parkinsonian symptoms and some marmoset specific symptoms. Additionally, quantitative assessment of animal behavior is an often-used tool. In MPTP non-human primate models it has been shown that measurement of changes in general activity has direct application to the clinic as hypokinesia is a common feature of Parkinson's disease. Furthermore, the fine motor skills of patients are reduced owing to a combination of tremor, slowless of movement and disturbed motor planning. Assessment of these fine motor skills in MPTP non-human primates, e.g. hand-eye coordination, is also affected (reviewed by Emborg, 2004). Finally, sleep aspects can be measured in these monkeys for the identification of the early signs of Parkinson's disease (Verhave et al., 2011).

5.4 Further research

Today, patients, family members and physicians struggle with the fact that there is no cure or satisfactory treatment for Parkinson's disease. The nature of the disorder is suggested to be a combination of endogenous and exogenous factors starting years before the actual diagnosis. Therefore, it is our notion that research should be directed towards a combination of early diagnosis and development of neuroprotective treatments. As a first step, a shift should be made from diagnosis that is completely based on motor complications (Pahwa & Lyons, 2010) to early diagnosis that is supported by premotor symptoms. Relatively simple but informative diagnostic tools are the Parkinson's disease Sleep Scale (Dhawan et al., 2006) and the University of Pennsylvania Smell Identification Test (Deeb et al., 2010). Furthermore, these tests can be combined with neuroimaging techniques for subjects at risk. It is known that pathological changes in the brain, such as dopamine transporter density, are already present before motor signs appear. Furthermore, the NAA/tCr ratio correlates with the metabolic state of a neuron. These changes can be measured with neuroimaging techniques in human as well as in marmoset monkeys. However, insight into the dynamics of potential diagnostic tools for the early phase of the disease and the mechanisms for neuropathology and subsequently neuroprotection in early neurodegeneration will rely on disease models. We found that an RBD-like phenomenon also takes place in marmosets with

moderate neurodegeneration. This underlines the fact that this mild MPTP model entails features of the premotor phase of Parkinson's disease. Therefore, the marmoset MPTP model for early Parkinson's disease offers a good opportunity to investigate neurodegeneration at different stages of pathology.

6. Conclusions

In order to halt or limit neurodegeneration, research should focus on a combination of early diagnosis supported by premotor symptoms and the further development of neuroprotective treatment. However, studying Parkinson's disease neuropathology related to disease manifestation in humans is limited to clinical trials and post-mortem material. In these circumstances, in the search for new targets for neuroprotective therapies, animal models represent a great asset in Parkinson's disease research. Insights into neuroprotection and the mechanisms of neuropathology in early neurodegeneration will rely on animal models to investigate the early stages of Parkinson's disease. Animal models should ideally mimic the main features of the disease pathology and additionally show the typical parkinsonian syndrome. The multi-factorial aspects of the disease dictate the need for Parkinson's disease-like animal models that couple disease manifestation to the underlying pathology.

The ultimate animal model for Parkinson's disease has not yet been developed; however, there are several experimental models used worldwide that reproduce Parkinson's disease-like neurodegeneration in combination with motor symptoms. One of these induction models is based on the neurotoxin MPTP that causes selective cell death in the dopamine neurons localised within the *substantia nigra* in humans, monkeys and mice, which indicate the high external validity. The marmoset MPTP model is congruent with the scientific criteria of face, predictive, construct and external validity. Besides motor deterioration and clinical parkinsonian signs, sleep and neuroimaging aspects can be relevant markers for moderate neurodegeneration in the marmoset MPTP model analogous to the clinical sleep problems and pathological changes in the brain, such as the REM sleep behavior disorder and affected NAA/tCr ratio, in the premotor phase of Parkinson's disease patients. Neuroimaging will give insight in the dynamic changes in the brain due to neurodegeneration and presents the opportunity to monitor the effect of a neuroprotective treatment over time. On the other hand, improvement in muscle tone during REM sleep can be seen as a novel diagnostic marker of early stages of Parkinson's disease and a useful marker for measuring neuroprotective effects in the marmoset disease model.

For the translation in the clinic, a combination of markers, such as genetic vulnerability, olfactory dysfunction, depression, RBD, neuroimaging abnormalities and slight motor signs, may be a helpful indicator for individuals at risk when developing Parkinson's disease. If treatment could be started at a stage when there are still neurons to protect, the quality of life of these patients could be improved.

7. Acknowledgment

8. References

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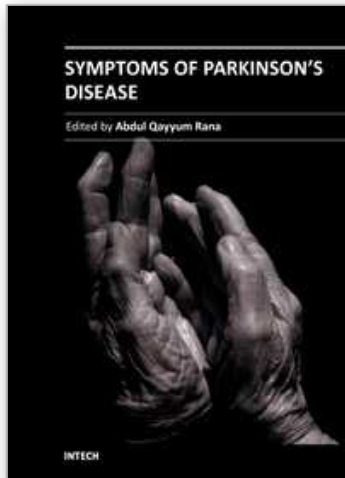
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This book about Parkinson's disease provides a detailed account of various aspects of this complicated neurological condition. Although most of the important motor and non-motor symptoms of Parkinson's disease have been discussed in this book, but in particular a detailed account has been provided about the most disabling symptoms such as dementia, depression, and other psychiatric as well as gastrointestinal symptoms. The mechanisms responsible for the development of these symptoms have also been discussed. Not only the clinicians may benefit from this book but also basic scientists can get enough information from the various chapters which have been written by well known faculty.

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