Systems pharmacology and blood-brain barrier functionality in Parkinson's disease

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Chapter 3

Animal Models as a Tool in Systems Pharmacology Research on Parkinson's Disease

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Abstract

Parkinson's disease is a progressive neurodegenerative disease, which is composed of many components, each caused by interplay of a number of genetic and nongenetic causes. This warrants a systems pharmacology approach to the development of novel drug treatments of Parkinson's disease. Animal models of disease are an essential asset in this research.

There is no single animal model which reflects all aspects of Parkinson's disease. The available animal models however may reflect specific aspects, making each animal model suitable for specific Parkinson's disease research questions. A combination of models may provide pertinent information on Parkinson's disease characteristics and treatment effects in the system as a whole.

Specifically, an animal model for Parkinson's disease displaying the slow progressive nature of the disease constitutes a basis to obtain the essential biological system-specific information to determine the neuroprotective properties of new drugs, preclinically.

In this review we have summarised the currently available animal models of Parkinson's disease, with their main characterstics, followed by a discussion on the *in vivo* monitoring technique microdialysis, to obtain information on the target site distribution. Here, the emphasis is on blood-brain barrier (BBB) functionality in Parkinson's disease and the added value of intracerebral microdialysis to assess disease related changes in BBB functionality affecting drug transport into and out of the brain, and the within brain distribution to the target site. Furthermore, to be able to determine the effect of drugs on the Parkinsonian behaviour in animal models, we have summarised the most commonly used behavioural tests used. Finally, the state of the art of mechanism PK-PD modelling is introduced as novel systems pharmacology approach to characterise drug effects *in vivo*.

1. Introduction

Parkinson's disease is a neurological disorder characterised by resting tremors, rigidity, bradykinesia and postural instability. These symptoms are a result of the loss of dopamine-producing neurons in the substantia nigra pars compacta (SNc) and a depletion of dopamine in the striatum. Clinical manifestations do not occur until there is a loss of approximately 50% - 80% of the dopaminergic neurons within the SNc and a loss of about 70% of the striatal dopamine (Bohlen Und, 2005; Dauer and Przedborski, 2003). A further histopathological hallmark of the disease is the presence of intracytoplasmic inclusions called Lewy bodies (LBs) in

the remaining dopaminergic neurons of the SNc. The major compounds in these eosinophilic LBs are aggregated forms of the normally presynaptically located protein α-synuclein (Bohlen Und, 2005; Dauer and Przedborski, 2003).
Today's treatment of the symptoms of Parkinson's disease consists predominantly of replacing the lost brain dopamine by means of the dopamine precursor L-DOPA (Mercuri and Bernardi, 2005), in combination with a peripheral decarboxylase inhibitor. To overcome motor complications associated with long-term treatment with L-DOPA, which occur in a later stage of the disease, L-DOPA is often administered in combination with either a dopamine agonist, a catechol-O-methyltransferase (COMT) inhibitor, or a monoamine-oxidase-B (MAO-B) inhibitor. However, the further the progression of Parkinson's disease the less effective symptomatic drug treatment becomes and the more complications occur. It is clear that there is a need for drugs that can reduce or even halt disease progression in Parkinson's disease by using drugs that have neuroprotective or neurorestorative properties (Bonuccelli and Del Dotto, 2006; Chen and Le, 2006; Schapira, 2008). Some of the drugs already in use as treatment in Parkinson's disease (e.g. dopamine agonists), or currently under investigation (e.g. A2A antagonists), seem to offer some neuroprotective benefits (Bonuccelli and Del Dotto, 2006; Olanow, 2009). However, to date no drug has been proven to be neuroprotective in Parkinson's disease (Olanow, 2009) or has been approved for a neuroprotective indication (Rascol, 2009). A major limitation in this respect is the lack of an endpoint that accurately reflects the underlying disease state (Olanow, 2009) while also no 'optimal' clinical trial design is readily available for studying neurprotective properties of drugs in Parkinson's disease (Rascol, 2009; Stocchi and Olanow, 2003). For estimating the actual treatment effect a clinical trial should allow the time course of treatment effects to be distinguished from those due to natural disease progression (Ploeger and Holford, 2008). Other obstacles include the absence of precise knowledge on the etiology and pathogenesis of Parkinson's disease. Parkinson's disease, like other neurodegenerative diseases, consists of many disease components, each caused by an interplay of a number of different genetic and nongenetic causes (Gasser, 2009).

Investigation on the interplay between these disease components and drug treatment efficiency cannot be studied *in vitro* alone, nor in the human situation. This implies the need for *in vivo* animal studies. Indeed, animal models of Parkinson's disease (or neurodegenerative diseases in general) have their restrictions, as there will be no animal model reflecting all aspects of this disease.

As a result it has been stated that there is a lack of relevant animal models (Kieburtz and Olanow, 2007). However, various animal models of Parkinson's disease reflect particular aspects of the disease, making each animal model suitable for specific Parkinson's disease research questions, while a combination of models may provide important answers on Parkinson's disease features and treatment effects in the system as a whole.

Mechanism-based pharmacokinetic-pharmacodynamic (PK-PD) models are useful here in the sense that they aim to characterise, in strictly quantitative manner drug effects *in vivo* under physiological and pathological conditions. To this end mechanism-based PK-PD models contain specific expression to characterise processes on the causal path between drug administration and response (e.g. target site distribution, target binding and activation, transduction/homeostatic feedback and diseases processes/progression). In the meantime it has been demonstrated that mechanism-based PK-PD models have excellent properties for extrapolation and prediction in systems pharmacology research (Danhof *et al.*, 2007). Systems pharmacology aims at the development of an understanding of the interactions between pathophysiology and drug action. To develop such an understanding it is necessary to analyse interactions across and between various scales of organisation (Wist *et al.*, 2009). To date most PK-PD modeling research has focused on healthy systems. A major challenge is the characterisation of drug effects on disease processes and disease progression. A particularly intriguing challenge is the prediction of drug effects on disease progression in man from preclinical research. For this, the development of chronically instrumented animal models to determine the time course of drug concentration at the target site along with the effect on disease progression is very important. An animal model for Parkinson's disease displaying the slow progressive nature of the disease is essential and would provide the necessary biological system specific information to determine the neuroprotective properties of new drugs, preclinically. Relevant biomarkers will enable a detailed and mechanistic description of disease progression and serve as useful tools in these disease models. Within the context of mechanism-based PK-PD modeling, a biomarker is defined as a measure that characterises, in a strictly quantitative manner, a process, which is on the causal path between drug administration and effect (Danhof *et al.*, 2005). However, a combination of biomarkers may be needed to provide a complete characterisation of the treatment effects (beneficial and harmful) or disease progression (Lesko and Atkinson, Jr., 2001).

In this review we have summarised the characteristics of the currently available animal models of Parkinson's disease, followed by a discussion on *in vivo* monitoring techniques to obtain information on the target site distribution (PK). Here, the emphasis is on blood-brain barrier (BBB) functionality in Parkinson's disease and on the effects of Parkinson's disease on drug transport into and out of the brain, and the within brain distribution to the target site. Furthermore, to be able to determine the effect (PD) of drugs on the Parkinsonian behaviour in animal models, we have summarised the most commonly used behavioural tests used. Subsequently, the main concepts of mechanism-based PK-PD modelling are presented and discussed.

2. Animal Models of Parkinson's Disease

Animal models are "experimental preparations developed in one species for the purpose of studying phenomena occurring in another species" (McKinney, 1984). Suitable research models display clear face validity (isomorphism), predictive validity (pharmacological correlation), and construct validity (homology and similarity in the underlying neurobiological mechanisms) (Fuchs and Fliugge, 2006). In the case of animal models of Parkinson's disease, the model should reproduce the main characteristics of the human disease, such as (1) selective lesion of dopaminergic neurons that evolves over time; (2) depletion of dopamine from the striatum; (3) presence of LBs in the remaining dopaminergic neurons, and (4) easily detectable motor deficits (Bohlen Und, 2005; Yuan *et al.*, 2005). Currently applied animal models for Parkinson's disease can be separated in toxin-induced models and genetic animal models (Meredith *et al.*, 2008).

Toxin-induced animal models

A common feature of all neurotoxin-induced models is that they affect mitochondrial function, either by inhibiting mitochondrial complex I or complex III (see Figure. 1) (Schober, 2004). The mechanisms through which systemic dysfunction of complex I produces neurotoxicity are as yet unknown (Uversky, 2004).

This review gives an overview of the commonly used neurotoxins in models for Parkinson's disease, namely 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tertahydropyridine (MPTP), paraquat, maneb and rotenone, as well as a number of less-known neurotoxins like reserpine, methamphetamine (METH) and proteosome inhibitors (PSI). A summary of the neurotoxin-induced models is given in Table 1.

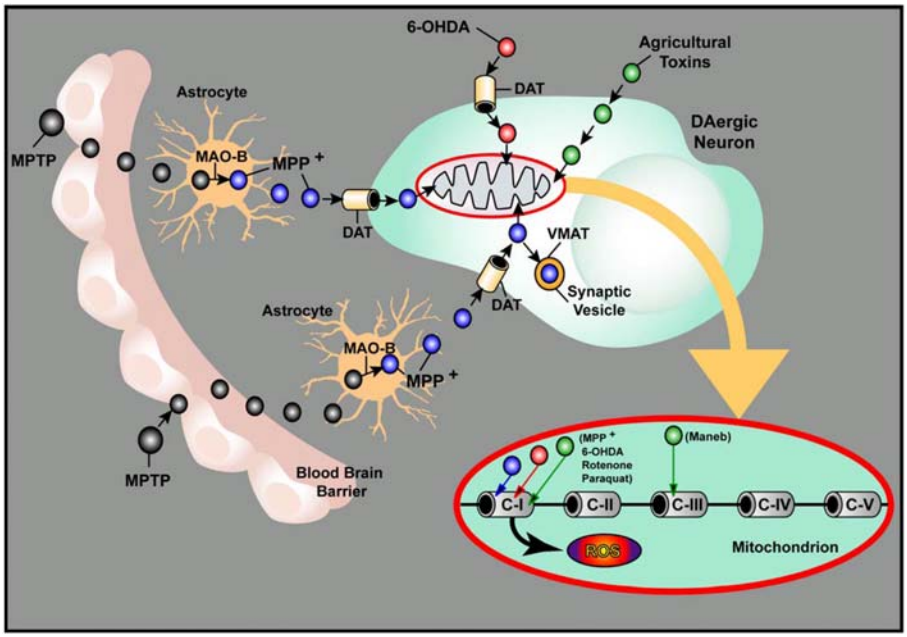

Figure 1: *Schematic overview of molecular and intracellular pathways of dopaminergic neurotoxins applied in animal models of Parkinson's disease. (taken with permission from Schober et al, 2004; Cell TissueRes 318:215-224)*

Reserpine

The administration of reserpine in rabbits was the one of the first animal models of Parkinson's disease. It caused a temporary slowness in movement that was reversed by the administration of L-DOPA (Carlsson *et al*., 1957). Reserpine is a naturally occurring alkaloid and when administered systemically to rodents, it is able to deplete dopamine at the nerve terminals and induce a hypokinetic state in the animals. However, since the effect of reserpine on the dopaminergic cells is temporary and striatal reserpine administration does not induce morphological changes in the nigral dopaminergic neurons, the model has limited use. Reserpine doesn't replicate the extensive biochemical and pathological changes seen in Parkinson's disease (Betarbet *et al*., 2002).

Methamphetamine

Methamphetamine (METH) is a psychostimulant which causes oxidative damage of nigrostriatal dopaminergic terminals (LaVoie and Hastings, 1999). In addition, METH-induced neurotoxicity may be linked to the glutamate and nitric oxide

systems within the striatum as it has been shown that glutamate antagonists are able to prevent METH-induced dopamine depletion (Kita *et al*., 2003; Sonsalla *et al*., 1989; Zeevalk *et al*., 1994). METH is an indirect catecholamine agonist, causing dopamine and norepinephrine release, and a potent inhibitor of MAO leading to an overall increase in cathecholaminergic activity in the brain (Green *et al*., 1992; Kita *et al*., 2003). METH presents an opportunity for modeling Parkinson's disease in that the neuropathology does overlap in terms of dopamine depletion, decreased tyrosine hydroxylase activity, loss of dopamine uptake sites and the loss of cell bodies in the SNc. However, there are no LBs present and striatal damage occurs in both the dopaminergic and serotonergic systems (Kita *et al*., 2003).

Table 1: *Neurotoxin-induced animal models of Parkinson's disease*

Neurotoxin	animal	How does it work?	LB	Acute/ Chronic	Applicable studies
Reserpine	Rat, mouse, rabbit	Systemic administration temporarily depletes DA. However, no morphological changes in SNc-DA neurons and it doesn't replicate typical biochemical and pathological changes.	NO	Acute	Effects of symptomatic therapies
METH	Rat, mouse, guinea pig, monkey	Oxidative damage of nigrostriatal DA terminals; DA and NE release and MAO inhibition leading to overall increase in brain CA. However, striatal damage occurs in both the dopaminergic and serotonergic systems.	NO	Acute	Mechanisms of pathogenesis
PSI	Rat, mouse	Striatal DA depletion and DA cell death with apoptosis and inflammation in the SNc. However data are not readily reproducible.	YES	Acute	Model under development
6-OHDA	Rat, mouse, monkey, cat	Selective DA cell death by oxidative stress and inhibition of mitochondrial complex I and IV. However, anterior olfactory structures, lower brain stem areas or the locus coeruleus are not affected.	NO	Acute (Chronic after intrastriatal injection)	Effects of symptomatic or neurorestorative therapies (neurprotective therapies with chronic model)
MPTP	Nonhuman primate, mouse, dog, cat, rat,	MPTP is converted to MPP+ which inhibits complex I in SNc-DA neurons. However, α-synuclein neurons in SNc were unlike typical LBs found in Parkinson's disease.	YES	Acute (Subchronic mouse model)	(1) Effects of symptomatic, neuroprotective, neurorestorative therapies (2) Mechanisms of pathogenesis
Paraquat/ Maneb	Rat, mouse	Reduces striatal TH and DA transporter immunoreactivity, striatal TH protein levels, and TH immunoreactivity and cell counts in the SNc. α-synuclein-positive inclusions in SNc neurons.	(YES)	Acute Subchronic	(1) Effects of symptomatic, neuroprotective, neurorestorative therapies (2) Role of environmental toxins in etiology
Rotenone	Rat, mouse	Rotenone denegerates SNc-DA neurons by inhibiting complex I and produces α-synuclein-positive LB-like inclusion. However, variable results and severe peripheral toxicity upon systemic administration.	YES	Chronic	(1) Effects of symptomatic, neuroprotective, neurorestorative therapies (2) Role of environmental toxins in etiology (3) mechanisms of pathogenesis

LB: Lewy Body; METH: Methamphetamine; PSI: Proteosome inhibitor; 6-OHDA: 6-Hydroxydopamine; MPTP: 1-methyl-4-phenyl-1,2,3,6-tertahydropyridine DA: dopamine; NE: norepinephrine; CA: cathecholamine

Proteosomal inhibitors

A relatively new rodent toxin-induced model in adult rats has been proposed that uses systemic administration of proteasomal inhibitors (PSI) such as epoxomicin. Animals treated with proteosomal inhibitors have been shown to develop behavioural symptoms like bradykinesia, rigidity, tremor, and an abnormal posture, which improved upon apomorphine treatment. Also, striatal dopamine depletion and dopaminergic cell death with apoptosis and inflammation in the

SNc has been observed. In addition, α-synuclein/ubiquitin-containing inclusions resembling LBs were present in some of the remaining neurons (McNaught *et al.*, 2004). Since the publication by McNaught, several laboratories have tried to reproduce these results; some groups with success, in rats or mice (Schapira *et al.*, 2006; Sun *et al.*, 2006; Zeng *et al.*, 2006), others have unfortunately failed (Bove *et al.*, 2006; Hirst and Ferger, 2008; Kadoguchi *et al.*, 2008; Kordower *et al.*, 2006; Landau *et al.*, 2007; Manning-Bog *et al.*, 2006; Mathur *et al.*, 2007). There could be various factors explaining these discrepancies between laboratories, i.e. manufacturing, storage, preparation and administration of PSI or animal-specific differences in the metabolism of the toxin (Hirst and Ferger, 2008; McNaught and Olanow, 2006). Furthermore, not much is known about the plasma and brain kinetics of PSI's (Hirst and Ferger, 2008). Conceptually the application of PSIs might be a good PD model, as proteosomal inhibition could work slowly and progressively like the disease. A prerequisite is to and a small molecule PSI (ideally for systemic administration), which enables a significant and effective proteasome inhibition in the nigrostriatal system (Hirst and Ferger, 2008).

6-OHDA

6-OHDA is the first animal model of Parkinson's disease associated with SNc dopaminergic neuronal death (Ungerstedt, 1968). 6-OHDA-induced pathology differs from Parkinson's disease as its induced toxicity is relatively selective for monoaminergic neurons, resulting from preferential uptake by dopaminergic and noradrenergic transporters (Luthman *et al.*, 1989). Other brain areas involved in Parkinson's disease, such as the anterior olfactory structures, lower brain stem areas or the locus coeruleus are not affected by 6-OHDA (Betarbet *et al.*, 2002; Del Tredici *et al.*, 2002). Inside neurons, 6-OHDA accumulates in the cytosol, where it induces cell death through oxidative stress and inhibition of mitochondrial complex I and IV (Figure 1) (Jeon *et al.*, 1995). As 6-OHDA is not able to cross the BBB, it must be administered by local stereotaxic injection into the SNc, median forebrain bundle (MFB; which carries ascending dopaminergic and serotonergic projections to the forebrain), or the striatum to target the nigrostriatal dopaminergic pathway (Dauer and Przedborski, 2003; Javoy *et al.*, 1976). Different models have been developed in mainly rodents and small monkeys (marmosets) using 6-OHDA, most of them unilaterally, resulting in a model that is commonly referred to as the "hemiparkinson model". At least in mice, rats, cats and primates, 6-OHDA is a highly effective toxin for dopaminergic neurons (Beal, 2001). Bilaterally affected animals cope with adipsia, aphagia and high mortality and

require intensive nursing care. In the hemiparkinson model, the contralateral side serves as an internal control, albeit that this side may also be influenced by the lesion, as is reflected in changes of striatal peptide and dopamine concentrations or changes in the electrical activity of neurons located in the subthalamic nucleus (Nieoullon *et al.*, 1977; Perier *et al.*, 2000; Salin *et al.*, 1996). Unilateral 6-OHDA injections into the SNc or the MFB cause an anterograde degeneration of the whole nigrostriatal dopaminergic system (O'Neill *et al.*, 2004; Sachs and Jonsson, 1975; Ungerstedt, 1968). Dopaminergic neurons start degenerating within 12-24 hr and die without apoptotic morphology. Striatal dopamine levels are depleted 2-3 days later (Faull and Laverty, 1969; Jeon *et al.*, 1995). After intrastriatal injection of 6-OHDA, it is taken up into the striatal terminals and causes a slow, progressive, induced neuron death, retrogradely, which lasts for 1-3 weeks (Murray *et al.*, 2003; Przedborski *et al.*, 1995; Sauer and Oertel, 1994). The magnitude of the lesion depends on the amount of 6-OHDA injected, the site of the injection, and the species used (Betarbet *et al.*, 2002). So far, none of the modes of 6-OHDA intoxication have led to the formation of LB-like inclusions (Dauer and Przedborski, 2003).

MPTP

MPTP currently represents the most important and most frequently used Parkinsonian toxin applied in animal models (Beal, 2001). MPTP is the byproduct of the unlawful manufacture of a synthetic meperidine derivative. Drug addicts who took MPTP accidentally developed a syndrome that clinically and pathologically resembled Parkinson's disease. After repeated injection of MPTP intravenously, chronic and severe parkinsonism developed, which was L-DOPA responsive. A tremor which was indistinguishable from the characteristic resting tremor occurred and neuropathological examination revealed moderate to severe depletion of pigmented nerve cells in the SNc, but an absence of LBs (Langston *et al.*, 1983). MPTP, which is a pro-toxin and highly lipophilic, crosses the BBB and is converted to 1-methyl-4-phenyl-2,3-dihydropyridium (MPDP) in non-dopaminergic cells (especially in astrocytes and serotonergic neurons) by MAO-B and then oxidixed to 1-methyl-4-phenylpyridinium (MPP+). Released from these cells, MPP+ is then actively transported into dopamine neurons where it impairs mitochondrial respiration by inhibiting complex I of the electron transport chain (Nicklas *et al.*, 1985; Vila and Przedborski, 2003). Endothelial cells in the microvasculature that make up the BBB contain MAO. Several studies have correlated levels of MAO with MPTP-induced neuronal loss (Kalaria *et al.*, 1987;

Riachi *et al*., 1988). Since MPP+ cannot be transported through the BBB (Riachi *et al.*, 1990), the BBB can be a first line of defense against exogenous agents (Smeyne and Jackson-Lewis, 2005).
MPTP is mainly applied in nonhuman primates and in mice but also in several other species such as dog, cat, rat and goldfish (Bohlen Und, 2005). With regard to the species used, several distinct routes of MPTP administration have been established such as oral or stereotactical injection, but also systemic administration by injection (e.g. subcutaneous, intravenous, intraperitoneal or intramuscular; (Przedborski *et al.*, 2001)) which is still the most common and reproducible form.

In contrast to primates, rodents are less sensitive to MPTP toxicity but the MPTP mouse model provides the most useful animal model of PD to study neuropathological and neurochemical changes (Schmidt and Ferger, 2001). In mice, systemic or intracranial administration of MPTP can result in severe damage of the nigrostriatal dopaminergic system, including symptoms such as akinesia, rigidity, tremor, gait and posture abnormalities. In MPTP-injected mice, a dramatic loss of dopaminergic neurons has been detected leading to impaired dopaminergic neurotransmission, which is accompanied by a loss of dopaminergic nerve terminals in the striatum and a dramatic reduction in striatal dopamine levels. The cell loss in the SNc is accompanied by an increase in the number of α-synuclein-immunoreactive neurons located in this brain area and an increase in α-synuclein mRNA (Vila *et al.*, 2000). However, when MPTP-treated animals developed α-synuclein inclusion bodies, these were not conform the distinctive architecture of LBs in Parkinson's disease (Maries *et al.*, 2003). Due to the insensitivity for MPTP, mice require relatively high doses to induce a significant loss of dopaminergic neurons resulting generally in acute pathology. Nevertheless, acute, low doses of MPTP are administered to mice to study compensatory mechanisms (Schmidt and Ferger, 2001). An acute treatment with a medial dose of MPTP induces a rapid dopaminergic degeneration with predominantly necrotic cell death (Schober, 2004) but for a subchronic induction of Parkinson's disease, medial doses of MPTP are injected 1-2 times a day for at least 5 days and for progressive chronic Parkinson's disease MPTP is given once daily at low doses over a time period of 20 days. The chronic MPTP-mouse protocol mirrors most closely the pattern of progression assumed to be that of Parkinson's disease and appears useful (Bezard *et al.*, 1997). Different mouse strains react very differently to MPTP. Systemic MPTP treatment of C57BL/6 and

BALB/c mice indicated a higher susceptibility to MTPT toxicity in C57BL/6 mice. No difference between gender was observed (Sedelis *et al*., 2000).

Rats are relatively resistant to MPTP (Terzioglu and Galter, 2008). Only injections of high doses of MPTP in rats which are therapeutically pretreated, e.g. with guanethidine, to prevent peripheral catecholamine release and extensive mortality, cause significant dopaminergic neurodegeneration. Rats are therefore not recommended for MPTP studies (Giovanni *et al*., 1994).

The MPTP monkey model remains the gold standard for the preclinical evaluation of new symptomatic therapies for Parkinson's disease (Dauer and Przedborski, 2003). The most commonly used administration mode in monkeys are multiple intraperitoneal or intramuscular injections as well as intracarotid infusions (Schober, 2004). In the past, primates were nearly always treated with high doses of MPTP to induce an acute and severe degeneration of dopaminergic neurons. However, views have changed and currently, monkeys are treated more and more with low doses of the neurotoxin for a prolonged period of time resulting for a more chronic degeneration and thereby mirroring the human pathogenesis more appropriately (Przedborski *et al*., 2001). Unilateral intracarotid artery (ICA) administration of MPTP and subsequent intravenous injections of MPTP in rhesus monkeys induces an advanced stable Parkinsonian syndrome, in which the ICA injection of MPTP initiates the Parkinsonian syndrome primarily in one hemisphere and the subsequent iv. doses (administered as needed) further deplete the dopamine system to induce a bilateral lesion in a shorter period of time, with fewer side effects (Oiwa *et al*., 2003). Although MPTP is able to mimick the features of Parkinson's disease well, other brain areas involved in Parkinson's disease, such as the locus coeruleus are not affected (Dauer and Przedborski, 2003). Moreover, continuous MPTP infusion by minipumps has been reported to induce development of intracellular inclusion bodies in baboons (Kowall *et al*., 2000), but these inclusions are not similar to the LBs typically found in human disease (Maries *et al*., 2003).

Paraquat and maneb

Exposure to environmental toxins has been associated with an increased risk for developing Parkinson's disease (Chade *et al*., 2006; Tanner, 1989). The herbicide paraquat (N,N′-dimethyl-4-4′-bipiridinium) and the fungicide maneb (manganese ethylenebisdithiocarbamate) are two pesticides which have shown to induce dopaminergic neuron degeneration in the SNc. Paraquat is structurally similar to MPTP, but has no selectivity for the dopamine transporter and therefore does not

accumulate in dopamine neurons after systemic administration. It does not easily penetrate the BBB but is possibly taken up into the brain by the L-neutral amino acid transport system, then transported into striatal, possibly neuronal, cells in a Na+-dependent manner (Shimizu *et al.*, 2001). Pretreatment of paraquat-treated mice with *L*-valine or *L*-phenylalanine seems to prevent neurodegeneration completely and thus confirms the uptake of paraquat into the brain by the neutral amino acid transporter (McCormack and DiMonte, 2003). Paraquat induces a specific, although modest, dose-dependent loss of striatal dopaminergic nerve fibres and SNc neural cell bodies (Brooks *et al.*, 1999; Terzioglu and Galter, 2008), although evidence in literature seems contradictory (McCormack *et al.*, 2002; Thiruchelvam *et al.*, 2000a; Thiruchelvam *et al.*, 2000b). The toxicity of paraquat appears to be mediated by the formation of superoxide radicals and the activation of cholinergic and glutamatergic transmission (Corasaniti *et al.*, 1998; Dauer and Przedborski, 2003). Subchronic treatment of rats with systemic paraquat significantly decreases brain dopamine content in the striatum and slightly in the midbrain and cortex and stimulates glutamate efflux from neural cells or inhibits the glutamate uptake system and thereby initiating a cascade of excitotoxic reactions (Shimizu *et al.*, 2003). Levels of α-synuclein were reported as elevated in both the frontal cortex and ventral midbrains. Finally α-synuclein-positive inclusions in the SNc neurons of mice treated with paraquat were observed (Manning-Bog *et al.*, 2002).

Much less is known about the mechanisms of maneb toxicity. Maneb consists of manganese and ethylene-bis-dithiocarbamate, both of these components being potentially neurotoxic. Which of the two components of maneb is responsible for the decreased locomotor activity and potentiation of the paraquat effects on the nigrostriatal pathway, remains to be clarified (Uversky, 2004). Paraquat in combination with maneb produces synergistic effects on the nigrostriatal dopamine system. Paraquat/maneb reduces striatal TH and dopamine transporter immunoreactivity, striatal TH protein levels, and TH immunoreactivity and cell counts in the SNc but not in the ventral techmental area (VTA) (Thiruchelvam *et al.*, 2000b). The effects of paraquat/maneb on the dopaminergic system are age-dependent. A greater decrease in dopamine turnover, dopamine metabolites, nigrostriatal dopaminergic neurons and striatal TH protein levels compared with younger mice as was shown in 18-month old mice compared with younger mice treated with paraquat/maneb (Thiruchelvam *et al.*, 2003). A study in 6-month-old rats also indicated an increased susceptibility of older animals to toxicity from paraquat/maneb compared to a study with

2-moth-old rats (Cicchetti *et al.*, 2005; Saint-Pierre *et al.*, 2006).
The animal models using paraquat alone or in combination with maneb are currently not applied for the evaluation of new therapies for Parkinson's disease. Thier use is limited to investigations into therelationship between exposure to environmental toxins such as paraquat and maneb and the development of Parkinson's disease in humans.

Rotenone
Another candidate for experimental work onthe role of environmental toxins in Parkinson's disease, is the pesticide, rotenone. Rotenone is the most potent member of the rotenoids, a family of natural cytotoxic compounds extracted from tropical plants and it is widely used as an insecticide and fish poison. Rotenone is highly lipophilic and readily gains access to all organs (Talpade *et al.*, 2000) and therefore it does not require the dopamine transporter to gain access to the neural interior. Rotenone binds (at the same site as MPP+) to and inhibits mitochondrial complex I, leading to a homogenous reduction of complex I activity throughout the brain. *In vitro*, subacute rotenone treatment of differentiated SH-SY5Y neuroblastoma cells resulted in death of approximately 60% of the cells. Furthermore, it appears to reproduce the Lewy neuritic changes of early Parkinson's disease pathology, although no LB inclusions were seen (Borland *et al.*, 2008). *In vivo*, the administration of low-dose intravenous or subcutaneous rotenone to rats produces selective degeneration of nigrostriatal dopaminergic neurons accompanied by α-synuclein-positive LB-like inclusions (Betarbet *et al.*, 2000; Sherer *et al.*, 2003). Rotenone can cross cell membranes and is therefore likely to affect all cells. However, rotenone seems to preferentially target dopamine neurons. These neurons are particularly sensitive to oxidative stress because of the permanent elevated level of free radicals generated by dopamine metabolism and auto-oxidation (Giasson and Lee, 2000; Olanow, 1990). However, differences were observed in rotenone vulnerability between different populations of dopaminergic neurons within the SNc, and between dopaminergic fiber projections and cell bodies (Betarbet *et al.*, 2000). In contrast to these findings, acute intoxication with rotenone seems to produce selective damage in the striatum and the globus pallidus, but spare dopaminergic neurons in the SNc (Ferrante *et al.*, 1997). The variability in the development of dopaminergic lesions in the brain of systemically administered rotenone as well as the high mortality rate have been reported previously (Betarbet *et al.*, 2000; Fleming *et al.*, 2004; Hoglinger *et al.*, 2003; Lapointe *et al.*, 2004; Zhu *et al.*, 2004). This high-variability

demonstrates the inter-individual susceptibility to rotenone, reflecting genetic differences among rats, providing insight into the interaction between environmental and genetic influences of Parkinson's disease pathogenesis (Perier *et al.*, 2003). Systemic administration of rotenone in rats seems to affect locomotor behaviour, however this can not be correlated to the degree of nigrostriatial injury seen in these animals. Most probably, changes in locomotion are a consequence of peripheral organ toxicity (Fleming *et al.*, 2004; Lapointe *et al.*, 2004;). These results were confirmed after repeated daily subcutaneous or oral doses of rotenone in C57BL/6 mice (Inden *et al.*, 2007; Richter *et al.*, 2007) although at high daily oral doses of 10 and 30 mg/kg a reduction of TH immunoreactivity in SNc was seen with variability being the lowest at the highest dose (Inden *et al.*, 2007). Interestingly, absorption of rotenone in the gastrointestinal tract is slow and incomplete and rotenone is effectively metabolised by the liver which would not favor the oral route for rotenone administration (Bove *et al.*, 2005). In another study systemic exposure of mice to rotenone demonstrated an acute increase in dopamine turnover which seems to be common to other mitochondrial toxins (Thiffault *et al.*, 2000).

It was demonstrated in a model of Drosophila melanogaster that L-DOPA is able to reverse rotenone-induced locomotor changes (Coulom and Birman, 2004) and a study with rats showed similar results whereby L-DOPA (in combination with a decarboxylase inhibitor) was able to reverse rotenone-induced catalepsy, postural changes and decreased locomotion, indicating that the behaviour was a consequence of the degeneration of dopaminergic neurons, rather than from systemic toxicity of rotenone. The rats were treated daily with low systemic doses of rotenone via i.p. administration for 60 days or rotenone was infused directly into medial forebrain bundle of the brain (Alam and Schmidt, 2004). These modes of administration cause a slow degeneration which makes it suitable to study neuroprotective agents (Schmidt and Alam, 2006).

Due to its inconsistent and unpredictable effect on the nigrostriatal pathway, the chronic (systemic) administration of rotenone is not yet suitable to become a routine animal model for Parkinson's disease (Bove *et al.*, 2005). Next to genetics (Perier *et al.*, 2003), various factors such as age (Phinney *et al.*, 2006; Richter *et al.*, 2007), rat species (Betarbet *et al.*, 2000; Schmidt and Alam, 2006) and even environmental temperature (Crutchfield and Dluzen, 2006) might contribute to the variability in results. An alternative approach is when rotenone is administered directly into the brain. Although this does not represent the natural exposure to environmental toxins, it might develop into an animal model which

could be applied in preclinical (PK-PD) studies in the search for neuroprotective strategies for Parkinson's disease.

Intracerebral-administered rotenone (2 µg dissolved in 5 µl 10% DMSO solution into the MFB) produced a decrease in dopamine and its metabolites in the striatum (about 70%) and SNc (35% for dopamine and 55% for DOPAC), without affecting the serotonin system (Antkiewicz-Michaluk *et al.*, 2004). Another study demonstrated that stereotaxic administration of rotenone into the MFB, SNc or striatum decreased dopamine levels in the striatum (96%, 62% and 30%, respectively). Furthermore, these animals showed behavioural changes to a similar degree as seen in 6-OHDA lesioned rats (Sindhu *et al.*, 2006). Intranigrally, rotenone-infused animals exhibited progressive ipsilateral rotations when challenged with amphetamine on days 7, 14, 21 and 28 whereas animals that received a MFB infusion of rotenone only displayed this behaviour on day 28 (Sindhu *et al.*, 2005). Thus, an infusion of rotenone into the MFB might develop a slower, more progressive degeneration of dopaminergic cells compared to an intranigral infusion. Moreover, the decrease in striatal dopamine on day 32 seems greater in MFB-infused animals compared with intranigrally infused animals (Sindhu *et al.*, 2005). In another study, an intranigral infusion caused a time-dependent reduction of complex-I activity, an increased production of hydroxyl radicals and a significant depletion of striatal dopamine in the ipsilateral SNc. No changes were seen in striatal serotonin levels. TH immunostaining revealed a highly significant decrease in the staining intensity in the striatum and an overt decrease in the area positive for TH in the ipsilateral SNc (Saravanan *et al.*, 2005). Bilateral infusion of rotenone into the MFB showed a strong increase in catalepsy, a decrease in locomotor activity and a significant depletion of striatal dopamine levels compared to sham-lesioned rats. L-DOPA is able to reverse the motor deficits in rats, confirming the depletion of dopaminergic neurons in these animals (Alam *et al.*, 2004). In our opinion, the intracerebral infusion of rotenone, specifically into the MFB, has the most potential as an animal model for mechanism-based PK-PD studies in search for treatments with neuroprotective properties.

Genetic animal models

The majority of Parkinson's disease cases (>95%) are sporadic, although some genes (associated with the PARK loci) have been identified and linked to rare forms of Parkinson's disease. Currently, there are six clearly defined genetic

causes of Parkinson's disease. There are α-synuclein (PARK1) and LRRK2 (PARK8), which result in autosomal dominant Parkinson's disease and Parkin (PARK2), DJ-1 (PARK7) and PINK1 (PARK6), which result in autosomal recessive Parkinson's disease (Pankratz and Foroud, 2007). The gene UCHL1 (PARK5) has been implicated but not confirmed.

For Parkin, DJ1 and PINK1, all of which cause early-onset Parkinson's disease, genetic mouse models can easily be made by null mutation of such genes (knockout mice). For the dominantly inherited gain-of-function mutations such as in α-synuclein (PARK1) and LRRK2 (PARK8), transgenic mouse models have been created in which extra copies of the gene are introduced into the mouse genome or delivered by lenti- or adeno-associated virus (Terzioglu and Galter, 2008). Since α-synuclein is a major component of LBs, and mutations in α-synuclein may result in nigrostriatal dopaminergic degeneration in familial Parkinson's disease it has gained the most focus in the development of transgenic mice or Drosophila flies, which express the wild-type or mutated α-synuclein. Transgenic mice overexpressing α-synuclein or expressing mutated forms of α-synuclein display a number of features seen in Parkinson's disease such as progressive accumulation of α-synuclein-and ubiquitin-immunoreactive inclusions in neurons in the neocortex, hippocampus, and SNc, mitochondrial DNA damage and degeneration, loss of dopaminergic terminals in the basal ganglia and motor impairments (Martin *et al.*, 2006; Masliah *et al.*, 2000). Similar results were found in rats overexpressing α-synuclein (Mochizuki *et al.*, 2006). Interestingly, several lines of α-synuclein null mice have a complete or partial resistance to MPTP (Dauer *et al.*, 2002; Drolet *et al.*, 2004; Schluter *et al.*, 2003). Animal models based on the transgenic expression of wild-type and mutated α-synuclein do not demonstrate all of the key features of Parkinson's disease, like a massive loss of dopamine nigrostriatal neurons (Betarbet *et al.*, 2002; Fleming *et al.*, 2005). Rapid neurodegeneration is observed after viral transduction but is limited to the targeted region and does not mimic the broad pathology observed in the disease (Chesselet, 2008). Nevertheless, they provide an important opportunity to study the involvement of α-synuclein in Parkinson's disease pathogenesis.

Other genetic models include the Parkin knockout mice, DJ-1 knockout mice, NURR1 and PITX3-APHAKIA, among others. The reader is referred to some excellent reviews for a more detailed description of these models (Fleming *et al.*, 2005; Terzioglu and Galter, 2008).

3. Measuring Target Site Distribution and BBB Functionality in Animal Models of Parkinson's Disease

The distribution of drug molecules between plasma and the target site in the brain is a crucial factor in the effects of drugs used in the treatment of Parkinson's disease. In the previous chapter it has been outlined that multiple mechanisms are involved in the distribution of drug molecules to the target site in the brain (i.e. brain perfusion, transport across the BBB, distribution within the brain). Moreover it has been demostrated that the functionality of the BBB may change under disease conditions. This underscores the need of studying the brain distribution kinetics of drugs used in the treatment of Parkinson's disease. In this pragraph we discuss principles and applications of intracebral microdialysis as a tool monitor the time course of extracellular brain concentrations.

Intracerebral microdialysis involves the insertion of a microdialysis probe into a selected area of the brain. The probe, consisting of a hollow tube and a semi-permeable membrane, is constantly perfused with a physiological solution. During perfusion, substances around the semipermeable part of the probe will diffuse from higher to lower concentration into the dialysate (Figure 2).

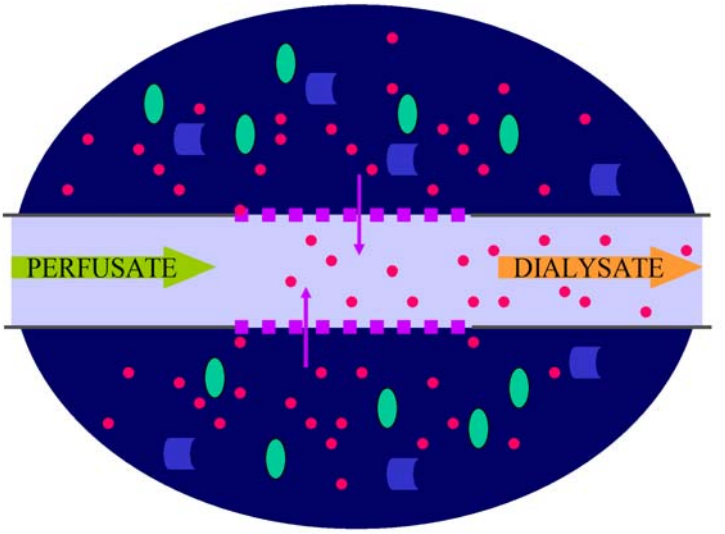

Figure 2: The principle of microdialysis

Drug concentrations in brain dialysate reflect but do not equal free (unbound) concentrations in brain ECF due to the constant flow of perfusion fluid (de Lange

et al., 1999). In vitro recovery, or better, in vivo recovery methods such as retrodialysis, no-net-flux and dynamic-no-net-flux need to be applied to be able to relate the concentration in the dialysate to the true concentration in the ECF (Bouw and Hammarlund-Udenaes, 1998; de Lange *et al.*, 1997; de Lange *et al.*, 1999).

Many studies using intracerebral microdialysis in experimental animals have already shown its usefulness and special value in monitoring brain pharmacokinetics. It has been shown that this technique providies better estimates of BBB transport parameters compared with the more classical tissue homogenate-pharmacokinetic approach (de Lange *et al.*, 1997; de Lange *et al.*, 1998; Hammarlund-Udenaes *et al.*, 1997; Hammarlund-Udenaes, 2000; Sawchuk and Elmquist, 2000; Wang and Welty, 1996; Xie *et al.*, 1999). A prerequisite for this application is that BBB transport characteristics are not significantly influenced by the microdialysis probe implantation and its presence in the brain. This was an initial concern but based on a series of studies performed to validate intracerebral microdialysis in the characterisation of passive, as well as active, BBB transport (de Lange *et al.*, 1994; de Lange *et al.*, 1995a; de Lange *et al.*, 1995b; de Lange *et al.*, 1998; de Lange *et al.*, 2000; Malhotra *et al.*, 1994; Ooie *et al.*, 1997; Wang and Welty, 1996), it has been demonstrated that this prerequisite holds, provided that the technique is used under well-controlled surgical and experimental conditions (de Lange *et al.*, 1997).

Intracerebral microdialysis is not only able to measure kinetics of exogenous compounds but also offers the possibility of monitoring endogenous compounds such as neurotransmitters and any changes in their kinetics as a consequence of a disease and/or its treatment (Baskaya *et al.*, 1997; Carter *et al.*, 1995; Frantz *et al.*, 2002; Fuchs and Hauber, 2003; Glick *et al.*, 1994; Hashiguti *et al.*, 1993; Jonkers *et al.*, 2001; Kaakkola and Wurtman, 1992; Napolitano *et al.*, 2003; Souza Silva *et al.*, 1997). Specifically in Parkinson's disease, intracerebral microdialysis has been applied to study the kinetics of L-DOPA and its metabolites in healthy and diseased rodents (Cannazza *et al.*, 2005; Fedrowitz *et al.*, 2000; Gerin, 2002; Giovanni *et al.*, 1994; Holmer *et al.*, 2005; Kannari *et al.*, 2006; Kostrzewa *et al.*, 2005; Marti *et al.*, 2000; Sarre *et al.*, 1992) or monkeys (Alexander *et al.*, 1994; Zhang *et al.*, 2003). Using intracerebral microdialysis, one is able to measure the PK of a (new) drug for Parkinson's disease and determine its distinct brain penetration properties and consequently measure its effect (PD) on brain neurotransmitters. Simultaneously, intracerebral microdialysis is able to measure a biomarker or a combination of biomarkers specific for Parkinson's disease in the same animal.

Furthermore, by means of dual probe microdialysis, one is able to measure compounds in different brain areas, in the same rat, at the same time (Galeffi *et al.*, 2003; Pudovkina *et al.*, 2002; Westerink *et al.*, 1998). Using mechanism-based PK-PD disease progression modeling, presumed neuroprotective properties of new treatments for Parkinson's disease can thus be investigated in a preclinical setting.

4. Measuring Behaviour and Drug Effects in Animal Models of Parkinson's Disease

To be able to assess lesion-induced disability and/or treatment-induced recovery, the appropriate but simple testing routines should be chosen. Behavioural tests should reflect histological and/or functional deficits or preservation of the injured tissue (Yuan *et al.*, 2005). Animal models of Parkinson's disease can be either unilateral as well as bilateral, requiring different behavioural assessments. The following section summarises behavioural tests which are commonly used in animal models of Parkinson's disease.

Rotometry

Rotational behaviour of unilateral-lesioned animals in response to dopamine receptor agonists has been the conventional method in the assessment of dopamine-mediated responses since the 1970s (Ungerstedt and Arbuthnott, 1970). Unilateral lesions (mainly by 6-OHDA) produce a hemiparkinsonian syndrome, which includes asymmetries of body posture, and contralateral sensorimotor deficits (Cenci *et al.*, 2002). The rotometry test consists of recording the number of turns that are performed by an animal after a challenge with dopamine agonists (amphetamine or apomorphine). The net rotational assymetry score is expressed as full body turns per minute (Moore *et al.*, 2001). Animals with unilateral dopamine depletion will turn contralaterally to the hemisphere in which dopamine-receptor stimulation is stronger; that is, they will turn towards the side of the lesion after challenge with dopamine-releasing drugs and away from it after treatment with L-DOPA or dopamine agonists (Yuan *et al.*, 2004).

Elevated body swing test

The elevated body swing test allows behavioural testing of unilateral lesioned animals without using drugs, to determine the natural response following a lesion (Roghani *et al.*, 2002). This test was first described by Borlongan (Borlongan and Sanberg, 1995) and adapted for various studies (Roghani *et al.*, 2002; Yuan *et al.*,

2004) including the assessment of body axis bias ('curling') (Henderson *et al*., 1999). The elevated body swing test involves elevating the animal by handling its tail and recording the frequency and direction of the swing behaviour. Unilateral nigral 6-OHDA-lesioned rats exhibited significant biased swing activity with the direction contralateral to the lesioned side, corresponding to the direction of apomorphine-induced rotations. A 30 seconds elevated body swing test was noted as the peak time for biased swing activity (Borlongan and Sanberg, 1995).

Head turning

In this test, the position of the head relative to the body axis is measured by placing the rat into a standard cage and allowing it to habituate. The position of the head (> 10 ° deviation left or right of the midline, or neutral) was noted every second for 60 seconds. The net number of seconds the head was positioned ipsilaterally minus the number of seconds positioned contralaterally is calculated over 3 min of observations (Henderson *et al*., 1999). From this, ipsilateral side bias and overall head-turning activity indices can be calculated (Moore *et al*., 2001).

Staircase test

The staircase test, also referred to in modified versions as the skilled paw reaching test, is a behavioural test that consists in reaching for food inside a special box and allows for a sensitive measure of skilled reaching by each limb in an independent manner (Montoya *et al*., 1991). This test is not only applied in unilateral-lesioned animals of Parkinson's disease, but aso in animals with experimental stroke, hypoxia and peripheral neuropathy (Pagnussat *et al*., 2009). The staircase apparatus comprises a test box from which runs a narrow corridor with a central plinth. On either side of the plinth is a descending 'staircase' of steps. Two food pellets are placed onto each step of two staircases located one on either side of the plinth. Rats are placed in the box and can reach down either side of a plinth to grasp lift and retrieve food pellets from the steps of the staircase. The numbers of pellets removed provides a quantifiable measure of the distance and efficiency of reaching skill. The design is such that the rat can only reach pellets on one staircase with its left paw and on the other with its right paw, thereby providing separate measures of performance for each limb (Jeyasingham *et al*., 2001; Kirik *et al*., 1998; Montoya *et al*., 1991; Moore *et al*., 2001; Pagnussat *et al*., 2009).

Stepping test

This test is a modification of the bracing test (Schallert *et al*., 1979). The rat is held

by the experimenter fixing its hindlimbs with one hand and the forelimb not to be monitored with the other, while the unrestrained forepaw is touching the table. The number of adjusting steps was counted while the rat is moved sideways along the table surface (90 cm in 5 s), in the forehand direction, for both forelimbs (Kirik *et al*., 1998; Olsson *et al*., 1995; Paille *et al*., 2007).

Grip strength

The grip strength test measures fore- and hindlimb grip-strength in rats or mice and is able to asses the effect of drugs, age and toxins on muscle strength (Meyer *et al*., 1979). This test was later modified to provide measurement of the two forelimbs, separately but simultaneously. A description of the apparatus is described elsewhere (Dunnett *et al*., 1998). Rats and mice naturally cling to the bars until they can no longer resist the pull, and then let go. The applied force at the point at which the rodent releases its grip for each paw is recorded by two spearate strain gauses connected to a digital readout (Jeyasingham *et al*., 2001).

Catalepsy test

Catalepsy in laboratory animals is defined as a failure to correct an externally imposed posture, so the typical catalepsy test consists of placing an animal into an unusual posture and recording the time taken to correct this posture (Sanberg *et al*., 1988). The most common catalepsy test is the 'bar test' where rats are placed with both forepaws on bars 9 cm above and parallel from the base and were in a half-rearing position. Latency time of the removal of the paw is then recorded (Nehru *et al*., 2008; Zhou *et al*., 2007). There are, however, many variations of this bar test as summarised elsewhere (Sanberg *et al*., 1988). Other tests applied to measure catalepsy are by using parallel bars, platforms or pegs to situate animals in unusual positions (Sanberg *et al*., 1988).

Rotorod

The rotorod (Rota-Rod) test, in which animals walk on a rotating drum, is widely used to assess motor status in laboratory rodents. Animals are pre-trained on the rotorod and then tested at a series of increasing rod speeds. This test is suitable for initial screening of lesions or therapies both in unilateral and bilateral lesioned animals (Rozas *et al*., 1997; Rozas and Labandeira Garcia, 1997). Performance is measured by the duration that an animal stays up on the drum as a function of drum speed. High-speed video recording methods of the animal walking on the rotorod gives information about qualitative aspects of walking movements

(Whishaw *et al*., 2008). Using the rotorod test, 6-OHDA-lesioned rats showed chronic impairment in their posture and in the use of the limbs contralateral to the lesion (Whishaw *et al*., 2003).

5. Mechanism-Based PK-PD Modelling Techniques

PK-PD modeling is increasingly applied in modern drug research. PK-PD modeling aims at characterisation and prediction of the time course of drug action in health and disease (Breimer and Danhof, 1997). In recent years PK-PD modeling has evolved from a descriptive discipline into a mechanistic science that is applied in all phases of drug discovery and development In this context advanced mechanism-based PK-PD models are now being developed, which have improved properties for *in vitro*/*in vivo*-, interspecies- and healthy volunteers to patients extrapolation and prediction.

Mechanism-based models

PK-PD has evolved from the basic concept of the dose-response relationship to sophisticated models enabling the understanding of the underlying mechanisms of drug action (Csajka and Verotta, 2006). Mechanism-based PK-PD models contain specific expression to characterise specific processes on the causal path between drug administration and response. These processes include: 1) target distribution, 2) target binding and activation, 3) transduction/homeostatic feedback and 4) disease processes/progression. As such mechanism-based PK-PD modeling utilizes concepts from physiologically-based pharmacokinetic (PBPK) modeling, receptor theory, dynamical systems analysis and disease systems analysis (Danhof *et al*., 2007). A key element of mechanism-based PK-PD modeling is the explicit distinction between the drug-specific and biological system-specific parameters. Drug-specific parameters (target affinity and target activation) can often be predicted on the basis of *in vitro* bioassays and are often identical between species and individuals, whereas biological system-specific parameters (PD interactions, time-dependent transduction mechanisms, homeostatic feedback mechanisms, disease processes and disease progression) can only be estimated by *in vivo* systems analysis and typically their values can vary between species, individuals, disease state and experimental conditions (Danhof *et al*., 2007; Danhof *et al*., 2008).

Interspecies scaling / allometry

In drug development many pharmacological and toxicological studies are performed in small laboratory animals such as mice, rats, rabbits, dogs and monkeys. Species differences and therefore interspecies scaling is an important issue for the prediction of pharmacokinetic parameters from these animals to humans. The assumption that animals have similar physiology, biochemistry and cellular structure has allowed interspecies scaling of heart rate, blood flow, blood volume and organ size (Mordenti, 1986). Clearance (Cl), V_d, and elimination half-life ($t_{1/2}$) are the three most frequently extrapolated pharmacokinetic parameters. Over the years, many approaches have been suggested to improve the prediction of these pharmacokinetic parameters in humans from animal data and consist of allometric methods (Mahmood, 1999) and PBPK models (Danhof *et al*., 2008; Rowland, 1985).

In short, the allometric approach is based on the power function Y = aWb, where the bodyweight of the species is plotted against the pharmacokinetic parameter of interest on a log-log scale. Using this approach, Cl is not predicted very well (error between predicted and observed clearance > 30%). Thus, several other approaches have been proposed, which have been described elsewhere (Feng *et al*., 2000; Fura *et al*., 2008; Mahmood and Balian, 1999; Sinha *et al*., 2008). The PBPK models provide a mechanistic-based evaluation of drug disposition in which they use a physiologically realistic compartmental structure (Danhof *et al*., 2008; De Buck *et al*., 2007) and therefore is a better approach for interspecies extrapolation as it uses species-specific data on tissue structure, volume and composition and on the associated blood flows (Danhof *et al*., 2008). The structure of the PBPK model is essentially common to all mammalian systems, thereby facilitating interspecies scaling, and these models are uniquely suited to predict tissue and organ exposure (Rowland *et al*., 2004).

Compared with the allometric approach, PBPK models also seem to have a higher percentage of successful predictions (Ito and Houston, 2005; Jones *et al*., 2006; Zuegge *et al*., 2001). PBPK modeling has been applied successfully to take species differences in functionality of transporters at the BBB into account and was used to predict target exposure of drugs such as selective serotonin re-uptake inhibitors and semi-synthetic opioids (Geldof *et al*., 2008; Groenendaal *et al*., 2007a; Groenendaal *et al*., 2007b; Liefaard *et al*., 2005).

Disease progression

In vivo systems analysis aims at the explanation of physiology and disease from the level of interacting components such as molecular pathways, regulatory networks, cells, organs, and ultimately the entire organism. In conventional PK-PD analyses, the values of these biological system-specific parameters in the absence of a drug are kept invariable with time, and physiology is generally considered constant at baseline. However, for progressive, chronic diseases like Parkinson's disease, this is not a realistic description as the biological functions (e.g. dopaminergic neurons) deteriorate over the time course of the (mostly symptomatic) treatment period. Therefore, disease progression analysis has been proposed where the influence of a drug effect on the change in disease status over time is characterised (Chan and Holford, 2001; Holford and Peace, 1992). In these first disease-progression models for clinical Parkinson's disease rating scales, the signs and/or symptoms of disease and their response to treatment are modeled directly, without consideration of the underlying biological system as only information on clinical symptoms or outcome was available.

A theoretical framework for mechanism-based disease progression models has been proposed, in which time-dependent changes in the biological system-specific parameters of diseased subjects are taken into account (Post *et al*., 2005). This is an important factor when searching for a drug treatment for Parkinson's disease intended to slow or stop the disease processes and disease progression. PK-PD modeling is increasingly applied in drug discovery and preclinical development i.e. in the selection of drug candidates with the most favorable PK and PD properties. For this purpose, the development of chronically instrumented animal models for the determination of the time course of drug concentration and effect is very important. An animal model for Parkinson's disease displaying the slow progressive nature of the disease would be able to provide the necessary information (biological system-specific parameters) needed to determine the neuroprotective properties of new drugs, preclinically. Availability of relevant biomarkers could result in a more detailed and mechanistic description of disease progression and serve as useful tools in these disease models. Within the context of mechanism-based PK-PD modeling, a biomarker is defined as a measure that characterises, in a strictly quantitative manner, a process, which is on the causal path between drug administration and effect (Danhof *et al*., 2005). However, a combination of biomarkers ("fingerprint") might be needed to provide a complete characterisation of the treatment effects (beneficial and harmful) or disease progression (Lesko and Atkinson, Jr., 2001).

6. Conclusions

As we have stated in the introduction, there is no single animal model which reflects all aspects of Parkinson's disease but one model may reflect specific aspects suitable for specific Parkinson's disease research questions. Specifically, an animal model for Parkinson's disease displaying the slow progressive nature of the disease constitutes a basis to obtain the essential biological system-specific information to determine the neuroprotective properties of new drugs, preclinically. In this review we have summarised the currently available animal models of Parkinson's disease. An animal model of Parkinson's disease should reproduce the main characteristics of the human disease, such as (1) selective lesion of dopaminergic neurons that evolves over time; (2) depletion of dopamine from the striatum; (3) presence of LBs in the remaining dopaminergic neurons, and (4) easily detectable motor deficits (Bohlen Und, 2005; Yuan *et al*., 2005).

Although all of the models display some means of damage to the nigrostriatal dopaminergic system (Table 1), causing a decrease of striatal dopamine, only the MPTP and rotenone models have shown to develop LB-like inclusion bodies. However, when MPTP-treated animals developed α-synuclein inclusion bodies, these were not conform the distinctive architecture of LBs in Parkinson's disease (Maries *et al*., 2003). MPTP, 6-OHDA and rotenone can be applied to induce a (sub)chronic disease progression. For MPTP, the chronic MPTP-mouse protocol mirrors most closely the pattern of progression assumed to be that of Parkinson's disease and appears useful (Bezard *et al*., 1997). In the case of 6-OHDA, an intrastriatal injection causes a slow, progressive, induced neuron death with the disadvantage that is is retrogradely and thus not displaying the normal disease progression which is from the SNc to the striatum and not vice versa. Given this, rotenone seems to be the best candidate for the development of an animal model for Parkinson's disease with a slow, progressive induction of the disease. Rotenone is usually administered systemically. Due to its inconsistent and unpredictable effect on the nigrostriatal pathway, the chronic (systemic) administration of rotenone is not yet suitable to become a routine animal model for Parkinson's disease (Bove *et al*., 2005). Next to genetics (Perier *et al*., 2003), various factors such as age (Phinney *et al*., 2006; Richter *et al*., 2007), rat species (Betarbet *et al*., 2000; Schmidt and Alam, 2006) and even environmental temperature (Crutchfield and Dluzen, 2006) might contribute to the variability in results. An alternative approach is when rotenone is administered directly into the brain. Although this does not represent the natural exposure to environmental toxins, it might develop into an animal model which could be applied in

preclinical (PK-PD) studies in the search for neuroprotective strategies for Parkinson's disease. Either way, rotenone was able to induce Parkinson's disease symptoms such as catalepsy, postural changes and decreased locomotion (Alam and Schmidt, 2004). These modes of administration cause a slow degeneration which makes it suitable to study neuroprotective agents (Schmidt and Alam, 2006).

An unilateral model using rotenone would create a model where the contralateral side serves as an internal control. Caution should be made, however, as this side may also be influenced by the lesion. Using intracerebral microdialysis, extracellular, unbound concentrations of endogenous compounds (e.g. biomarkers for Parkinson's disease or BBB functionality) as well as of exogenous compounds (e.g. antiparkinson drugs or marker compounds for specific transport mechanisms of the BBB) could be quantified simultaneously at various time point during the progression of the disease. Any, or a combination, of the behavioural tests may be applied for the unilateral lesioned animal model although one should always take the influence of a microdialysis probe on the behaviour into consideration, if applicable.

A bilateral model using rotenone (administration either systemically or intrecerebrally) would give a more realistic representation of the human disease progression and behaviours. A separate control (sham-lesioned) group of animals would be needed for comparison of $brain_{ECF}$ concentrations of endogenous or exogenous compounds as well as for the behavioural experiments. In the latter case, not all of the summarised tests as described here, can be applied. The grip strength test, the catalepsy test and the rotorod test are suitable for use in the bilateral animal model. The staircase test may be applied to determine the difference in the total amount of food pellets taken between control and lesioned rats, irrespective of which paw was used. Similarly with the stepping test, where the number of total adjusting steps could be counted between control and lesioned rats, again, irrespective of which paw was used.

The data obtained from these experiments give information on processes on the causal path between drug administration and response (e.g. target site distribution, target binding and activation, transduction/homeostatic feedback and diseases processes/progression). With this, mechanism-based PK-PD models can be developed which can have properties for extrapolation and prediction in systems pharmacology research (Danhof *et al*., 2007). Systems pharmacology aims at the development of an understanding of the interactions between pathophysiology and drug action. To date most PK-PD modeling research has

focused on healthy systems. The use of a chronic animal model for Parkinson's disease is a first step in the characterisation of drug effects on disease processes and disease progression. The next particularly intriguing challenge is the prediction of drug effects on disease progression in man from this preclinical research.

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