Summary
Parkinson’s disease (PD) is one of the main chronic and progressive neurodegenerative disorders characterized by extensive loss of dopaminergic neurons, in particular in the substantia nigra (SN). Most patients are in the prime of their life, when they start suffering from the classic symptoms of motor dysfunction. To relieve their symptoms these patients are dependent on dopamine (DA) replacement treatment. Before the display of motor deterioration, they report non-motor problems, such as olfactory dysfunction, mood changes and sleep problems that can start years before the actual diagnosis. Although the ultimate clinical goal is to limit neurodegeneration, this is only possible by an early identification of individuals at risk and early start of neuroprotective treatment before progressive loss of neurons is achieved. Therefore, this research project was initiated as a response to the increasing interest in early PD and the initiation of the neurodegenerative process based on the hypothesis that neuroprotection at an early stage of PD will limit its progression and that protection will reduce the functional deterioration and pathology. This thesis aims to give additional insight in functional and molecular markers of DA neurodegeneration using animal models for PD.

In chapter 1, PD is introduced as a multi-factorial disorder, caused by the combined effect of age, environmental factors, genetic susceptibility and complex genetic-environmental interactions. The processes affected by these factors are: mitochondrial dysfunction, glutamate excitotoxicity, oxidative stress, inflammatory responses and proteasome dysfunction. Finally, this results in a loss of dopaminergic neurons in the SN. These neurons are the key players in the motor domains, responsible for the fine-tuning of movements. At the time of diagnosis, which to date is mostly based on the classic motor symptoms, PD patients have already lost at least 50% of the DA neurons in the SN. Together with the loss of these neurons the amount of released DA is reduced.

Unfortunately, there is still no cure or satisfactory treatment for PD that can stop the neurodegenerative process due to the multi-factorial nature of PD and the slow induction process. The initiation of the disorders is caused by a combination of endogenous and exogenous factors, which lead to neurodegeneration already years before the actual diagnosis based on symptomatology. Therefore research efforts should be focused on a combination of markers for early diagnosis and neuroprotective treatment.
Studying PD 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, animal models are a great asset in PD research. 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 PD-like animal models that couple disease manifestation to the underlying pathology. The ultimate animal model for PD has not yet been described; however there are several experimental models used worldwide that model PD-like neurodegeneration in combination with motor symptoms. One of these induction models is based on the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), the compound of choice for this thesis, which causes selective cell death in the DA neurons highly specific for the SN in humans, monkeys and mice.

In Chapter 2 we present two new clinically relevant biomarkers for DA neurodegeneration in an animal model closely related to humans. Two new behavioral test systems were developed for marmoset monkeys to quantify jumping behavior as a measure for akinesia (Tower test) and the righting reflex as a measure for rigidity and axial turning (Hourglass test). The marmoset’s righting reflex in the Hourglass test remained significantly impaired during the period after the MPTP intoxication. In the Tower test, the marmosets were not able to jump the largest distances one week after MPTP and showed a persistent reduction in activity after the MPTP intoxication. Because not all aspects of motor behavior are similarly affected by MPTP, a complete behavioral sketch of Parkinsonian marmosets should preferably include a range of motor behavioral functions to create an overview of the full range of motor impairments. Both the Hourglass and Tower test provide important behavioral information in a clinically-relevant approaches for testing motor dysfunction.

Besides the motor function tests, symptoms related to sleep are evaluated as a possible marker for moderate neurodegeneration in the marmoset MPTP model in chapter 3. This is a relevant parameter for the clinical sleep problems in the premotor phase of PD. We describe changes during rapid eye movements (REM) sleep, analogous to REM sleep behavior disorder (RBD) in patients; one of the suggested premotor symptoms of PD. MPTP increased the number of epochs with high-amplitude muscle contractions during
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REM sleep. Of all sleep measures, RBD-like measures discriminated best MPTP-treated versus control animals. At this stage of sleep disturbances, the marmoset monkeys did not show any changes in functional motor behavior as measured by the Hand-eye coordination test. This REM sleep specific change, in the absence of profound changes in wake motor behavior, suggests that the MPTP marmoset model is of high potential for studies into mechanisms and treatment of RBD and sleep disturbances in the early phase of PD.

To validate neuroprotection in early PD models the anti-excitotoxic compound riluzole was tested for its neuroprotective efficacy in marmosets in chapter 4 and in mice in chapter 5. Riluzole is effective in ALS because of its life prolonging effects. However, riluzole appeared to be less or completely ineffective in clinical trials when started in the motor phase of PD. Cell loss can only be prevented if started before or at the same time as the actual initiation of the apoptotic process. Therefore, the effect of pretreatment with the riluzole is examined in chapter 4 in the MPTP-treated marmoset model for the early phase of PD. Not only the traditional observational scoring and histo-pathological evidence for neuroprotection is included in the analysis, but also a complete range of motor behavioral tests and sleep aspects, in order to find the most efficient way of predicting the therapeutic value of a compound in a preclinical trials. MPTP affected all behavioral parameters and sleep architecture and induced a relatively mild (50%) decline of DA neurons in the SN. Riluzole relieved the Parkinsonian signs, and improved the hand-eye coordination as well as turning ability. Moreover, riluzole prevented the impact of MPTP on sleep architecture and RBD. Riluzole also improved the number of surviving DA neurons. However, riluzole did not prevent the MPTP-induced impairments on locomotor activity and jumping activity. In conclusion, reduction of excitotoxicity by riluzole appeared to be effective in reducing progressive neurodegeneration and in minimizing several clinically relevant PD symptoms in an animal model representing the early phase of PD.

Insights into the multiple effects produced by riluzole on MPTP-challenged neurons and their synaptic transmission can also help us to understand why this drug might be clinically useful. In chapter 5 the effect of riluzole was evaluated for the early molecular changes of neurodegeneration by means of proteomics analysis in the synaptosome of DA neurons. The changes in protein expression in the brain cannot be measured in humans for medical
ethic reasons. Besides this, the actual start of the neurodegenerative process cannot be estimated or predicted for idiopathic PD. Therefore the changes in protein expression were investigated in a chronic low level MPTP mouse model. MPTP was slowly infused in mice to generate a chronic progressive cell challenge. By sampling brain tissue at different time points, neurodegenerative as well as neuroprotective processes on protein expression were studied directly after the initiation of DA neuron specific neurodegeneration. The low dose 24 h MPTP challenge used in this mouse study did not affect the number of neurons and it did not lead to behavioral abnormalities. On the other hand, mitochondrial proteins showed a general increase of abundance at 24 hours after the start of infusion, reflecting either upregulation of mitochondrial activity or the recruitment of mitochondria into the synaptic fraction. Riluzole treated MPTP-exposed mice did not show this upregulation at 24 hours. This rescue effect of riluzole suggests a general inhibition of the mitochondrial activity in the synapse, which may act protective against the MPTP challenge. We showed the mitochondrial dysfunction at the start of a cell challenge, suggesting that this is the key factor in DA neurodegeneration and an important focus for further research. This relevant mechanistic and neuroprotective information could be used to delay or perhaps even arrest the disease before the typical symptoms emerge and the damage due to the neurodegenerative state becomes irreversible.

Today, patients, family members and physicians struggle with the fact that there is no cure or satisfactory treatment for PD. Therefore in chapter 6 we emphasize that research should focus on a combination of early diagnosis supported by premotor symptoms and the further development of neuroprotective treatment. Insights into neuroprotection and the mechanisms of neuropathology in early neurodegeneration will rely on animal models for the early stage of PD. Both the marmoset MPTP model and the mouse MPTP infusion model used for this thesis are congruent with the scientific criteria of face, predictive, construct and external validity. The general conclusion is that the specific scientific question raised should match the model and that the two models used in this study offer a good opportunity to investigate neurodegeneration at different stages of pathology. The behavioral tests and the RBD-like sleep changes in the marmoset monkeys add to the range of PD hallmarks in the marmoset model. The protective effect of riluzole on tonic muscle tone during REM sleep implies an important contribution of riluzole in the treatment of
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early stage PD. Improvement in tonic muscle tone during REM sleep can be seen as a novel diagnostic marker of the early stage neuroprotection. However, direct translation of features of the marmoset model to a clinical situation remains a rather difficult challenge because of the chemical induction of PD used, the natural recovery in marmosets from MPTP intoxication and the involvement of non-motor pathways in marmosets’ measured motor behavior. The increased abundance of mitochondrial proteins in the early stage PD-like MPTP mouse model demonstrates that this is a main mechanism by which neurons cope with this neuronal challenge. The validation with riluzole in this thesis raises the insight in the possibilities for riluzole as a treatment against early neurodegeneration. In conclusion, the etiology of idiopathic PD is suggested to be influenced by a combination of endogenous and exogenous factors starting years before the actual diagnosis. Therefore, this thesis emphasizes that research should focus on new means of early diagnosis and the development of neuroprotective treatments during the early phase of PD.