Chapter 1

General introduction
Parkinson’s disease

Parkinson’s disease is a neurodegenerative disorder named after James Parkinson, who first described the disease as the ‘shaking palsy’: a chronically progressive disorder with mainly motor symptoms. In the past two centuries a more elaborate picture of the disorder has emerged. Nowadays, we define Parkinson’s disease (PD) as a multisystem disorder characterized by the key motor symptoms: bradykinesia, rigidity, tremor and postural instability; and a wide range of non-motor symptoms. The non-motor symptoms of PD occur throughout the disease and include autonomic dysfunction, sleep-wake disorders, sensory disturbances and neuropsychiatric disorders. The clinical profile of motor and non-motor symptoms can vary considerably between patients. This clinical heterogeneity is a complicating factor both in the differential diagnosis and the choice of treatment.

The aetiology of the disease in the most common, idiopathic form is not well understood. Post-mortem histopathological analysis provides confirmation of the clinical diagnosis and offers a view on the pathological processes at the cellular level. The neuropathological characteristics of PD include intracellular alpha-synuclein accumulations, called Lewy bodies and neurites, and the loss of pigmented dopaminergic cells in the substantia nigra pars compacta (SNc). Based on the distribution of the histopathological changes, Braak et al. [1] developed a staging system for PD pathology, classifying a progressive spread of Lewy body pathology from the brainstem to the neocortex over the course of the disease. Neuropathological studies have revealed that cellular pathology can be detected in the parkinsonian brain even (years) before the clinical diagnosis of PD. Nigral neuronal loss seems to be an early phenomenon, followed by the appearance of alpha-synuclein aggregates [2, 3] illustrating the chronic development of the disorder at the histopathological level. It is important to note that the present pathological concept of PD is not restricted to the neurotransmitter dopamine, but also involves noradrenergic, serotonergic and cholinergic systems [4].

In spite of the dynamically changing concepts about the underlying pathological processes in PD, the gold standard therapy for the classical motor symptoms to date is dopamine replacement therapy. Unfortunately, chronic treatment with levodopa can induce motor response fluctuations and involuntary movements (dyskinesias). Almost thirty years ago, a novel therapeutic approach was introduced to alleviate the motor symptoms of PD: deep brain stimulation (DBS) [5, 6]. DBS is an invasive intervention that involves functional neurosurgery where a stimulator electrode is implanted in the basal ganglia (in most cases the subthalamic nucleus or the internal segment of the globus pallidus [7]) that can be activated by an external neurostimulator. High-frequency stimulation of the subthalamic nucleus (STN) alleviates parkinsonian symptoms and usually allows a reduction in the dosage of dopamine replacement therapy. In addition, during electrode placement
recordings of brain activity can be performed to study basal ganglia function in vivo in human subjects [8]. This type of experiment allows us to investigate the function and dysfunction of the basal ganglia in connection with the neocortex. Several parallel basal ganglia thalamocortical circuits have been defined that are associated with various functions (the limbic, oculomotor, prefrontal and motor loops) [9, 10]. The present thesis focuses on the circuitry associated with motor activity. Briefly, the traditional model of basal ganglia function distinguishes three pathways that lead from the cortex through the basal ganglia: first, the ‘direct pathway’ involves the striatum and the internal segment of the globus pallidus (GPi)/substantia nigra pars reticulata (SNr) complex, and leads back to the cortex through the thalamus; second, the indirect pathway runs though the striatum, the external segment of the globus pallidus (GPe), and the subthalamic nucleus to join the ‘direct pathway’ at the level of the GPi/SNr; third, the ‘hyperdirect pathway’ forms a direct connection between the cerebral cortex and STN, from where it feeds into the indirect loop [11, 12]. In the dopamine-depleted condition (in parkinsonism) the balance between these pathways is disturbed, the indirect and hyperdirect pathways being overactive and inhibiting thalamic neurons, thus constraining motor activity. In addition, the pro-kinetic effects of the direct pathway are diminished as a result of the dopaminergic depletion [11, 13]. Although recent findings challenge the above-presented classical model of basal ganglia function, it provides a comprehensive view of the complex interplay between basal ganglia and cortex in motor processing [9, 14, 15].

**Animal models of Parkinson’s disease**

To gain a better understanding of the pathophysiological mechanisms involved in PD at the level of the basal ganglia animal models have been developed. These models provide an opportunity to study various deep brain structures linked to PD and to investigate the changes induced without the confounding effects of chronic medication. Various approaches have been used in mammals to mimic PD: application of neurotoxins to induce nigrostriatal neurodegeneration; induction of alpha-synuclein pathology by transgenic modifications or by means of viral-vectors; development of genetic models [16, 17]. Neurotoxin models have been most widely studied and are known to present the relevant clinical motor features of PD: 1-methyl-4-phenyl-1, 2,3,6-tetrahydropyridine (MPTP) in primates and 6-hydroxydopamine (6-OHDA) in rats. The latter compound induces selective dopaminergic cell death in the brain after local (intracerebral) application [18]. In the classic hemiparkinsonian rat model, 6-OHDA is applied to the medial forebrain bundle in a single hemisphere, to reach the dopaminergic cells of the substantia nigra. In spite of the unilateral administration of the toxin, 6-OHDA lesioned rats present many features of PD, and do not suffer severe complications of the neurotoxin (unlike cases injected bilaterally) [16, 19]. Therefore the unilateral 6-OHDA model is an appropriate
candidate for investigating basal ganglia thalamocortical circuitry in the dopamine-depleted state.

**Neural oscillatory activity**

In the past century neuroscientists and neurophysiologists have developed various tools to shed light on the functioning of the human brain in vivo. Early measurements of brain electrical activity with the first electroencephalogram (EEG) already revealed rhythmic patterns of the recorded signal. The coordinated activity, the periodic co-activation patterns that occur over time in neurons or neuronal populations are called ‘neuronal oscillations’ [20, 21]. Neuronal activity can be investigated using a number of techniques, which vary largely by their temporal and spatial resolution of acquiring signals. Some imaging techniques, such as functional magnetic resonance imaging (fMRI) or positron emission tomography (PET), provide a good spatial resolution, but less accurate temporal resolution when measuring human brain activity. Superior temporal resolution can be achieved at the expense of spatial resolution or restrictions to the number of measurable areas with non-invasive tools such as EEG and magnetoencephalography (MEG). In specific medical conditions neuronal activity can be recorded from the cortical surface during brain surgery (electrocorticography: ECoG), or from deep brain structures in patients implanted with electrodes for deep brain stimulation (e.g. from the basal ganglia). The latter option offers a special opportunity to map the function of human deep brain structures; however data can only be acquired in patients suffering from chronic neurological or psychiatric disorders. Animal models enable us to investigate multiple deep brain structures both in the intact and pathological conditions. In addition, in an animal model more specific manipulations of neurotransmitters and/or distinct neuronal populations are possible through the application of pharmacological agents or optogenetics [22]. Local field potentials (LFPs) are a measure of the electrical activity of a neuronal population surrounding the inserted electrode. This results in continuous signal, similar to that of EEG and MEG. Another possibility in animal models is the analysis of brain activity at the cellular level in vivo, or in vitro, with the option to record and manipulate neuronal activity as detailed as the single cell or single ion channel level [21, 23]. In this thesis, recording of LFPs is used as a tool to investigate the activity of a local neuronal population in the brain, since it has similar properties to the non-invasive clinical tools to record brain activity (EEG, MEG).

Data analysis of continuous, high time resolution signals, such as EEG, MEG, or LFP offers the opportunity to examine local or more distant synchronized oscillatory patterns. The complex signals that can be recorded from the brain consist of multiple frequencies and evolve over time. To characterize the different components of the underlying activity, the frequency and time domain of the signal have to be dissected. For an overall picture of local neuronal activity, the signal can be described in the frequency
domain: the power spectrum reflects the amplitude of the recorded signal for each given frequency. The frequency spectrum is generally divided into a number of distinct frequency bands: delta (1.5–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), beta (13–30 Hz) and gamma (30–80 Hz), fast (80–200 Hz) and ultra-fast (200–600 Hz). As a measure of the synchronization between oscillatory signals recorded over different brain areas, the statistical interdependencies between the signals recorded over the brain regions can be computed. The degree of synchronization of recorded brain activity is taken as a measure of functional interconnectedness or ‘functional connectivity’ between the brain areas. A common measure of functional connectivity is coherence. Other methods can alleviate the spurious effects of volume conduction: e.g. the phase lag index (PLI) [24, 25]. To map the directional influences between two brain regions, ‘effective connectivity’ measures, such as Granger causality can be used. The latter method estimates how much of the activity in one brain area might influence the activity pattern of the other region of interest [26].

Synchronized neural oscillations and neural oscillatory networks play a role in numerous physiological processes, including attention, sensorimotor processes, visual processes, memory, motor control and sleep [20, 27–32]. Disturbances of neuronal synchronization patterns have been described in several neuropsychiatric disorders: schizophrenia, epilepsy, multiple sclerosis, Alzheimer's disease, and Parkinson’s disease [30, 33–36].

**Neuronal oscillatory patterns in Parkinson’s disease**

Resting-state measurements of EEG and MEG signals have been used to describe the changes in functional brain activity associated with PD. MEG recordings in PD patients have revealed a widespread, diffuse slowing of cortical oscillatory brain activity, detected even at the earliest clinical stages of the disease, and developing further in more cases and patients with PD dementia [37–41]. In addition to the presence of changes in functional brain activity at the level of the cerebral cortex, PD is characterized by changes in basal ganglia activity: there is increased beta oscillatory activity in the STN [42–45]. Resting-state oscillatory brain activity in PD may also serve as biomarker of the underlying disease process, and can predict later conversion to dementia in non-demented PD patients [46, 47].

Resting-state functional connectivity between cortical areas in PD was found to be increased and dynamically changing over the course of the disease: increases in alpha-band connectivity in the early stages, and enhanced beta-band connectivity in more advanced stages [34, 48–50]. Longitudinal analysis revealed dynamically changing connectivity patterns associated with disease progression: the increased synchronization in the alpha band that was present in de novo patients was reversed at later stages of the disease [50]. In patients undergoing functional neurosurgery, where basal ganglia recordings are available,
increased beta-band functional connectivity was found between cortex and basal ganglia (in most cases STN) [44, 51–53]. The increased beta-band activity is considered as a hallmark of dopamine depletion in PD, as treatment (dopaminergic medication or high frequency DBS) can both alleviate symptoms and cause a decrease in local and interregional coupling of beta activity [48, 54–60].

In addition to resting-state brain activity, task-related activity, in particular movement-related activity is of great interest in PD [61]. Motor activity can reduce the local and interregional beta synchronization patterns observed in PD patients [54, 55, 62, 63]. Also, during movement, activity develops in various brain areas [53, 64]. Investigations on movement initiation, which is typically disturbed in PD, showed a disturbed pattern of event-related desynchronization of beta activity and event-related synchronization of gamma activity [65–68]. These observations were summarized in the hypothesis of beta activity having an ‘anti-kinetic’ and gamma activity a ‘pro-kinetic’ effect [69, 70]. Recently, the detrimental role of excessive beta oscillations has come to the focus of attention: although a causal relationship between increased beta activity and parkinsonism is not evident, its contribution to the pathophysiology of PD is supported by numerous experiments [58–61, 71, 72]. Taken together, neuronal oscillatory activity is dynamically changing throughout the different stages of PD, both between components of the basal ganglia–cortex circuits and between different behavioural conditions.

Neuronal oscillatory patterns in the hemiparkinsonian rat model

In the present thesis, the objective was to investigate how changes in EEG-like synchronization patterns develop as a consequence of the loss of dopaminergic neurons in the hemiparkinsonian rat model. Numerous studies have demonstrated that the hemiparkinsonian rat model reflects several neurophysiological characteristics found in PD patients. Local oscillatory activity (spectral power) is elevated in the beta band in awake as well as anaesthetized rodents, both in the cortex and the basal ganglia (including striatum, STN, GPi, SNr) [73–80]. Previous studies revealed disturbed cortico-striatal and cortico-subthalamic information processing in the parkinsonian state [81–85]. It is important to mention that some of these experiments were conducted in animals under anaesthesia, which might limit extrapolation to awake subjects (humans or animals) [78]. In awake animals, synchronization patterns between cortex and basal ganglia are similar to what has been described in patients [73, 77, 79]. A few studies explored how local oscillatory activity and coupling patterns change as the dopaminergic cell loss in the midbrain develops [74, 79]. Research to date in animal models was mostly focused on a single hemisphere. Therefore, a number of issues remain unresolved. First of all, it remains to be explained how the changes in interhemispheric coupling known to occur in PD patients [48–50], how it develops over the course of dopaminergic degeneration. Another interesting issue is how
the different elements of the basal ganglia cortical loop drive each other in a situation of dopamine depletion, in other words, whether there are detectable changes in directional influences between various brain areas associated with parkinsonism. Some experiments suggest an increased cortico-basal ganglia drive, however the available evidence is far from consistent [77, 81, 86]. Taken together, the hemiparkinsonian rat model reflects the increase in local beta activity and the excessive cortico-subcortical beta synchronization seen in PD patients, and therefore appears to be a suitable model to investigate the changing patterns of functional brain activity in more detail.

**Aim, objectives and outline of the thesis**

The overall aim of this thesis is to describe the changes in synchronized neuronal oscillatory brain activity within and between cortex and basal ganglia in the hemiparkinsonian 6-OHDA rat model, including the temporal aspect of the development of these changes over time. To address this aim we had the following specific objectives:

1. Develop a freely-moving unilateral 6-OHDA rat model that will enable serial recordings simultaneously from several brain structures of the basal ganglia-cortex circuitry in awake, unrestrained, behaving animals.

2. Describe the effects of a unilateral 6-OHDA lesion on oscillatory brain activity in bilateral motor cortical areas and the subthalamic nucleus, and to assess the changes in functional connectivity between these areas.

3. Delineate the time course of changes in oscillatory brain activity and functional connectivity in bilateral motor cortical areas and the subthalamic nucleus in response to a unilateral 6-OHDA lesion.

4. Assess the extent of the changes in oscillatory brain activity and functional connectivity induced by a unilateral 6-OHDA lesion using simultaneous recordings from multiple cortical areas.

5. Study the changes in directional influences among brain areas after a unilateral 6-OHDA lesion.

6. Describe to what extent the neurophysiological characteristics in the hemiparkinsonian rat model resemble those observed in PD patients.

To achieve these objectives we developed a freely-moving 6-OHDA rat model that enabled us to record LFPs simultaneously from multiple cortical and subcortical brain areas before, during and after the development of dopaminergic cell loss in the substantia nigra, and in two behavioural conditions: at rest and during locomotion. To enable chronic data collection from the same brain areas throughout the course of the experiments, we designed two different recording devices. Using the first device (chapter 2) we registered LFPs from
motor cortices bilaterally and the ipsilateral STN in rats with a unilateral 6-OHDA lesion. We used serial intraoperative recordings to target the STN, and once the estimated depth and desired STN-like neurophysiological parameters [8] were obtained, the electrodes were fixed. The second device (chapters 3 and 4) was designed to collect LFP signals from multiple, relatively distant cortical areas, somewhat similar to the recording of an EEG in human subjects. To attain this, we designed a 3D printed electrode holder device that enables simultaneous recordings from up to 14 brain areas including cortical and striatal sites. Both recording devices were designed and implanted in a way that enabled regular recordings for more than 5 weeks after surgery, throughout the development of the nigrostriatal dopamine depletion. In chapter 2 we describe how neurophysiological parameters of brain activity in the bilateral motor cortex and ipsilateral STN (i.e. local oscillatory activity, interregional synchronization and directionality) alter in response to a unilateral dopaminergic cell degeneration in the rat induced by 6-OHDA. More specifically, we report on the changes in cortico-subthalamic activity and the source of these alterations in ‘time’ (by mapping changes throughout the development of dopaminergic cell loss) and ‘space’ (by computing directionality between the investigated brain areas). In chapter 3, we report the changes in local oscillatory activity, functional connectivity, and effective connectivity using the 14-electrode device in the 6-OHDA-lesioned rat. In chapter 4, we analyse the data derived from the 14-electrode recording in the unilateral 6-OHDA-lesioned rat using methods that were originally developed for human EEG recordings to determine to what extent changes in functional connectivity resemble those observed in PD patients. Chapter 5 provides a summary and a comprehensive discussion of the results presented in this thesis, including directions for future studies.
REFERENCES

Introduction

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