Biochemical and pharmacokinetic studies in vivo in Parkinson’s disease

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Linköping 2013
To Emmy and Eric!

Utan tvivel är man inte klok
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ABSTRACT

Parkinson’s disease (PD) is a neurodegenerative disease affecting approximately 25 000 people in Sweden. The main cause of the disease is the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc) projecting to the striatum. The motor symptoms of PD, due to decreased levels of dopamine, includes bradykinesia, rigidity and tremor.

During the 1960ies oral L-dopa treatment was introduced increasing quality of life for PD patients. In recent decades, enzyme inhibitors have been introduced, increasing bioavailability of L-dopa in plasma. After 5-10 years of L-dopa treatment, 50% of PD patients develop disabling dyskinesias. This can be due to rapid changes in L-dopa concentrations with non physiological stimulation of the dopamine receptors.

For over 20 years deep brain stimulation (DBS) has grown to become a routine neurosurgical procedure for improving quality of life in advanced PD with disabling dyskinesias. With stereotactic technique, electrodes are implanted in the brain and connected to a pacemaker sending electrical impulses. The most common target in PD is the subthalamic nucleus (STN). The knowledge about DBS mechanism(s) and its interaction with L-dopa is unsatisfactory.

The aims of this thesis were; to study the effect of the enzyme inhibitor entacapone on the L-dopa concentration over the blood brain barrier (BBB); to study possible interactions between L-dopa and DBS; to study alterations in neurotransmitters during DBS; to visualize microdialysis catheters in anatomical targets and to estimate sampling area of the catheters.

In all four papers the microdialysis technique was used. It is a well-established technique for continuous sampling of small water-soluble molecules within the extracellular fluid space in vivo, allowing studies of pharmaceutical drugs and neurotransmitters.

We showed that entacapone increased the bioavailability of L-dopa in blood with a subsequent increase of L-dopa peak levels in the cerebrospinal fluid. This in turn may cause a larger burden on the dopaminergic neurons causing an increased degeneration rate and worsening of the dyskinesias; we showed that 18% of L-dopa crosses the BBB and that there is a possible interaction between L-dopa and DBS, L-dopa concentrations increase during concomitant STN DBS, which can clarify why it is possible to decrease L-dopa medication after DBS surgery. The research has also showed that STN DBS had an effect on various neurotransmitter systems, mainly L-dopa, dopamine.
and GABA. We showed that STN DBS might have a direct effect on the SNC, resulting in putaminal dopamine release.

We showed that, it is possible to perform microdialysis sampling in specific areas in the brain with stereotactic technique. Simulations with the finite element method combined with patient specific preoperative MRI and postoperative CT images gave us exact knowledge about the positions of the catheters and that the studied structures were the intended. The research has given an assumption of the maximum tissue volume that can be sampled around the microdialysis catheters.
LIST OF ORIGINAL PAPERS

This thesis is based on the following papers, which will be referred to by their roman numerals:

I  M. Nord, P. Zsigmond, A. Kullman, K. Arstrand, N. Dizdar

The effect of peripheral enzyme inhibitors on levodopa concentrations in blood and CSF
Mov Disord. 2010 Feb 15;25(3):363-7

II P. Zsigmond, D. N. Dernroth, A. Kullman, LE. Augustinsson, N. Dizdar

Stereotactic microdialysis of the basal ganglia in Parkinson’s disease


A model for simulation and patient-specific visualization of the tissue volume of influence during brain microdialysis

IV P. Zsigmond, M. Nord, A. Kullman, E. Diczfalusy, K. Wårdell, N. Dizdar

Neurotransmitter levels in basal ganglia during levodopa and DBS treatment in Parkinson’s disease
Submitted 2013
### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>AC</td>
<td>Anterior Comissure</td>
</tr>
<tr>
<td>AUC</td>
<td>Area Under Curve</td>
</tr>
<tr>
<td>AADC</td>
<td>Aromatic L-amino acid decarboxylase</td>
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<tr>
<td>ADL</td>
<td>Activities of Daily Living</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood Brain Barrier</td>
</tr>
<tr>
<td>BG</td>
<td>Basal ganglia</td>
</tr>
<tr>
<td>CMAX</td>
<td>Concentration maximum</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<tr>
<td>COMT</td>
<td>Catechol-O-methyltransferase</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>DBS</td>
<td>Deep brain stimulation</td>
</tr>
<tr>
<td>FEM</td>
<td>Finite element method</td>
</tr>
<tr>
<td>GABA</td>
<td>Gammabutyric acid</td>
</tr>
<tr>
<td>GPe</td>
<td>Globus pallidum externa</td>
</tr>
<tr>
<td>GPi</td>
<td>Globus pallidum interna</td>
</tr>
<tr>
<td>HFS</td>
<td>High frequent stimulation</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptophan (serotonin)</td>
</tr>
<tr>
<td>i.v.</td>
<td>intravenous</td>
</tr>
<tr>
<td>L-dopa</td>
<td>Levodopa</td>
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<td>LID</td>
<td>L-dopa induced dyskinesia</td>
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<tr>
<td>MAO-B</td>
<td>Monoamine oxidase-B</td>
</tr>
<tr>
<td>MSA</td>
<td>Multiple System Atrophy</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
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<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>PD</td>
<td>Parkinson’s disease</td>
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<tr>
<td>rTVI_{max}</td>
<td>radius of the Maximum tissue volume of influence</td>
</tr>
<tr>
<td>SMA</td>
<td>Supplementary motor area</td>
</tr>
<tr>
<td>SN</td>
<td>Substantia nigra</td>
</tr>
<tr>
<td>SCN</td>
<td>Substantia nigra pars compacta</td>
</tr>
<tr>
<td>SNr</td>
<td>Substantia nigra pars reticulata</td>
</tr>
<tr>
<td>STN</td>
<td>Subthalamic nucleus</td>
</tr>
<tr>
<td>TVI_{max}</td>
<td>Maximum tissue volume of influence</td>
</tr>
<tr>
<td>UPDRS</td>
<td>Unified Parkinson’s Disease Rating Scale</td>
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<td>Zi</td>
<td>Zona incerta</td>
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Introduction

INTRODUCTION

Parkinson’s Disease

Parkinson’s disease (PD) is a neurodegenerative disease and the typical mean age of onset is 55-60 years. It occurs in 1-2% of persons over the age of 60 years (Tanner and Aston, 2000) and 0.3% of the general population is affected by PD. In Sweden approximately 25 000 people have the diagnosis PD. The prevalence of PD is higher among men than women with a ratio of 1.6:1 (Fahn 2003). The disease is characterized by the motor symptoms tremor, bradykinesia, rigidity, postural instability and gait disturbances. PD also has a multitude of non-motor manifestations including depression, memory difficulties, dementia and sleeping disorders. Many patients develop autonomic dysfunctions including digestive problems and orthostatic problems (Okun 2012). These symptoms can have a tremendous negative effect on the patients’ quality of life. PD is diagnosed clinically by the findings of distal tremor, bradykinesia, rigidity and an asymmetrical onset of the disease. In order to be diagnosed with PD the patients must respond to levodopa (L-dopa) medication or dopamine agonists.

Pathophysiology

The pathophysiology in PD is complex, involving multiple motor and non-motor neural circuits in the basal ganglia (BG). The believed main cause of the disease is the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc) projecting to the striatum (mainly putamen). Dopaminergic depletion in hypokinetic disorders such as PD can be explained as an increased activity in the BG output nuclei, the Globus pallidum interna
(GPi) and Substantia nigra pars reticulata (SNr). The onset of the clinical manifestations of PD is preceded by a period over several years in which the progressive loss of the dopamine innervation of the striatum is asymptomatic. Different stages of the pathophysiology have been proposed correlating morphological changes in the brain with clinical symptoms (Braak et al., 2004).

**Medical treatment**

Today no cure exists for PD patients and the therapies including medical and surgical treatment are aimed to minimize the clinical symptoms and to maintain and increase quality of life for PD patients.

To assess the progression of the disease and the response to the treatment a standardized assessment tool called the Unified Parkinson’s Disease Rating Scale, UPDRS is used. The UPDRS is a protocol divided into four parts that are used for documentation of (1) mental effects, (2) limitations in activities of daily living, (3) motor impairment and (4) treatment or disease complications.

Treatment for the early stage of PD is started at the onset of functional impairment. The treatment of PD is based on dopaminergic therapy, which is very effective in counteracting the motor disability. The most common pharmacological agents for treating motor symptoms are L-dopa, which is metabolized to dopamine and dopamine agonists, which mimic the effect of dopamine and activate the dopamine receptors. Other drugs are enzyme inhibitors acting on aromatic L-amino acid decarboxylase (AADC), monoamine oxidase (MAO-B) and catechol-O-methyltransferase (COMT), thus inhibiting the degradation of both levodopa, in peripheral blood, and dopamine, in the central nervous system (CNS), see figure 1.

Entacapone is a selective COMT inhibitor acting in the periphery with minor effects in the CNS. The COMT inhibitors increase the level and
bioavailability of L-dopa but have no own effect and need to be combined with L-dopa medication. The combination of COMT inhibitors and L-dopa enables a reduction of the L-dopa dose by up to 20-30% but with maintained mean L-dopa concentration in plasma.

![Figure 1](image)

**Figure 1.** The figure illustrates the different enzymes involved in degradation of L-dopa and dopamine. The main effect of COMT inhibitor Entacapone and the AADC inhibitor Carbidopa are in the periphery and the effect of the MAO-B inhibitor Selegiline is in the basal ganglia, resulting in increased dopamine concentrations.

**Side effects**

L-dopa is the single most effective treatment for all cardinal features in PD and initially has consistent therapeutic effects. Over time as a consequence of the interaction between disease progression and the effects of the long-term medication, the duration of the symptomatic benefit produced by each dose of dopaminergic therapy tends to decrease. This phenomenon is called wearing-off and can occur in up to 50% of patients within the first years of therapy.
(Martinez-Martin and Hernandez, 2012). The phenomenon is well characterized in terms of the reappearance of motor symptoms such as bradykinesia, rigidity and tremor.

**Figure 2.** The figure illustrates the wearing off phenomenon. (A) shows the therapeutic window in the early phase of treatment. The arrows indicate intake of tablets. (B) after time the therapeutic window decreases resulting in a gap with non adequate levels of L-dopa. This results in the need to administer the drug with shorter interval to bridge the gap (grey arrow).

Approximately 50% of the patients develop L-dopa induced dyskinesias (LID), after approximately 5 years, when the patients are in a progressive stage of the disease (Stoessl 2010). It often involves hyperkinetic movements, such as chorea, dystonia, and athetosis. L-dopa induced dyskinesias may be divided into various clinical forms: (1) “Peak-dose” dyskinesias related to high plasma levels of L-dopa. “Peak-dose” dyskinesias are choreatic movements involving the neck, trunk and upper extremities but dystonic movements may also occur. (2) Diphasic dyskinesias appears at the onset and offset of the L-dopa effect characterized by repetitive and stereotyped slow movements of the lower extremities and upper extremity tremor. (3) “Off” period dystonia is characterized by fixed and painful postures more frequently affecting the feet (Guridi and Gonzalez-Redondo, 2012).
Two main factors are involved in the origin of L-dopa induced dyskinesias: (1) degree of dopaminergic nigrostriatal depletion and (2) the pharmacokinetics and action of L-dopa, which delivers a discontinuous or pulsatile stimulation of the dopaminergic receptors. This can induce plastic synaptic abnormalities in striatal neurons altering physiological activity of striato-pallidal circuits leading to the abnormal pattern of neuronal activity underlying L-dopa induced dyskinesias (Guridi and Gonzalez-Redondo, 2012).

**Surgical treatment**

**Stereotaxy**

Stereotactic surgery is a minimally invasive form of neurosurgery using a three dimensional coordinate system to locate small targets in the brain prior to electrode insertion. The method is used in surgical treatment of PD.

The stereotactic method was initially developed by Victor Horsley and Robert H. Clarke in Britain and called the Horsley-Clarke apparatus. It was specifically designed for use in animal experiments. Later, in the 1940es the American neurologist Ernest Spiegel and the neurosurgeon Henry Wycis developed a stereotactic apparatus for use in the human brain. They used intracerebral reference points e.g. the posterior commissure to localize targets.

During recent years, several different stereotactic systems have been developed.

The Swedish neurosurgeon Lars Leksell designed the Leksell stereotactic arc system, with the goal of making it easy to work with in clinical work. The Leksell stereotactic system® (Elekta Instrument AB, Sweden) is now used worldwide and is used at all Neurosurgical Departments in Sweden.
Stereotactic ablative (lesioning) surgery has been used for many years in treating patients with movement disorders. Gradually the ablative surgery has declined, mainly due to the fact that it is an irreversible method.

Today many of the anatomical and target structures for stereotaxy can be directly visualized with radiologic methods like CT and MRI. Also several stereotactic atlases are available to help in calculating different target positions in the brain (Schaltenbrand 1977, Morel 2007, Talairach 1988).

**Figure 3.** The figure illustrates the Leksell Stereotactic System® which uses a three-dimensional reference system and center-of-arc instrument positioning, enabling neurosurgeons to localize target areas in the brain with high accuracy. Courtesy of Elekta AB, Sweden.
Deep brain stimulation

Deep brain stimulation (DBS) is a neurosurgical treatment involving the implantation of electrodes in the brain. The DBS operations are performed with the help of stereotaxy to localize the targets. The system consists of three components; a neurostimulator, an extension cable and a quadripolar lead, see figure 4. The neurostimulator is battery powered and sends electrical impulses to the brain interfering with neuronal activity. The neurostimulator is programmed by specially trained PD nurses, neurologists or neurosurgeons.

The first DBS treatment with STN stimulation in Sweden was performed in the beginning of the 1990ies. Today it is used routinely in PD, essential tremor and in dystonia. DBS is also used in the treatment of severe chronic pain and of various affective disorders such as depression, obsessive-compulsive disorders and Tourette syndrome. The most frequently used target area in Parkinson’s disease is the subthalamic nucleus (STN).

The device sends programmable high frequency electrical impulses to the stimulated area and usually there are prompt therapeutic benefits for the patients. One of the benefits of STN DBS is that the medication with L-dopa can be decreased and thereby the L-dopa induced side effects can be postponed. Long-term follow-up studies in patients with PD and STN DBS have confirmed the effectiveness in improving LID and activities of daily living (ADL) several years after surgery (Moro et al., 2010, Rodriguez-Oroz et al., 2012). A recent study has shown that subthalamic stimulation is superior to medical therapy in patients with PD presenting early motor complications with respect to motor disability, ADL, LID and “on” time with good mobility and no dyskinesia (Schuepbach et al., 2013).
Figure 4. (A) Illustrates an implanted DBS system consisting of brain electrodes, extension cables and pulsegenerators. Courtesy of Medtronic. (B) Fluoroscopy image of the quadripolar DBS electrode in target during a stereotactic operation. The electrode contacts are arranged in the following order, from distal to proximal; 0,1,2,3. The proximal electrode contact is indicated by the arrow. (C) Illustrates the pulsegenerator, Activa PC. The pulsegenerator measures 5 x 6 x 1 cm.

**Patient selection**

The outcome of surgical treatment with DBS in Parkinson’s disease is highly dependent on appropriate patient selection. The most important factor is that the diagnosis of idiopathic PD is confirmed prior to proceeding with DBS surgery. Patients accepted for DBS surgery need to have a diagnosis of idiopathic PD presenting “on-off” fluctuations with shortened “on” time and good L-dopa responsiveness (Kramer et al., 2010). It is generally considered
that a younger patient with less severe disease and with good L-dopa response will have the most favourable outcome of the surgery.

Several other neurological disorders might mimic the signs and symptoms of idiopathic PD. Multiple system atrophy (MSA) and progressive supranuclear palsy (PSP) are two differential diagnoses that must be considered (Machado et al., 2006).

Patients referred for DBS surgery must be thoroughly evaluated by a multidisciplinary team preferably consisting of a neurologist, neurosurgeon, neuropsychologist, specially trained PD nurse, occupational therapist, speech therapist and physiotherapist.

**DBS-mechanisms and hypothesis of action**

The mechanism of action is largely unknown, debated and not very well understood. The following neural responses have emerged as plausible explanations:

**Somatic activity in the stimulated nucleus (Depolarization blockade hypothesis)**

The earliest hypothesis on DBS action stated that DBS inhibits neuronal activity in the stimulated site leading to decreased output from the stimulated site, similar effects as after surgical lesion (Benabid et al., 1987). Meissner showed that high frequency stimulation (HFS) of the STN in monkeys decreased the mean firing rate in the majority of STN neurons (Meissner et al., 2005). Proposed mechanism(s) behind a reduction of somatic activity near the nucleus are depolarization block due to an increase of potassium current, inactivation of sodium channels, pre synaptic depression of excitatory afferents and stimulation induced activation of inhibitory afferents (Shin et al., 2007, Johnson et al., 2009). Magarinos-Ascone et al. demonstrated that HFS
could depolarize the membrane potential and trigger the action potential that subsequently lead to total silence of cells within the STN. They suggested that the silencing effect of tetanic stimulation is not due to a frequency dependent presynaptic depression but rather from the gradual inactivation of Na+ mediated action potentials (Beurrier et al., 2001, Magarinos-Ascone et al., 2002). This hypothesis is mainly built on *in vitro* experiments. *In vivo* experiments have shown that the somatic inhibition may not apply to all neurons surrounding the active DBS electrode and that a small number of STN neurons exhibit higher firing rates during HFS (Tai et al., 2003). In summary, this hypothesis states that HFS induces a functional ablation by suppressing the activity of the hyperactive target structure.

**Axonal output of the stimulated nucleus (“Output activation hypothesis”)**

The inhibition of the somatic activity of the stimulated nucleus described above may not necessarily parallel the output of the stimulated nucleus. Several experimental studies suggest that output is increased from an ostensibly inhibited nucleus, bringing into question the mechanism underlying this paradoxical dissociation (Hashimoto et al., 2003). One explanation of the mechanism can be that when a cell is exposed to extracellular stimulation, the stimulus-induced action potential initiates in the axon rather than in the cellbody. HFS could in this way increase output from the stimulated site and change the firing pattern and mean discharge rate of neurons at the projection sites (Johnson et al., 2009). This is shown in animal studies by Hashimoto, who demonstrated that in primates the neuronal firing rates in GPe and GPi increased in response to therapeutic HFS of the STN suggesting increased output from the STN (Hashimoto et al., 2003, Johnson et
al., 2009). Human studies with PET have shown that the blood flow in the GPi region is increased during HFS STN which would be consistent with activation of output from the stimulated site (Hershey et al., 2003). The output activation hypothesis has been shown in other experiments, e.g. Anderson et al. described in primates that HFS of the GPi inhibited thalamic neurons which is consistent with orthodromic activation of GABAergic projections. This could interrupt abnormal patterns of thalamic discharge associated with parkinsonian symptoms (Anderson et al., 2003). The increased output to downstream nuclei is corroborated by evidence from neurochemical measurements in animals and humans. In humans Stefani et al. have done microdialysis studies showing that cGMP, a secondary messenger in the glutaminergic pathway, increase in the GPi and SNr during STN DBS. The same group have shown that L-dopa and DBS reduces the GABA content in the motor thalamus with a subsequent activation of the thalamocortical loop. (Stefani et al., 2006, 2011, Galati et al., 2006, Fedele et al., 2001). Other microdialysis studies in mainly rats detected both elevated levels of glutamate in SNr and GP which also is consistent with increased STN output (Windels F et al., 2000, 2003). It has been suggested that neurochemical effects of HFS are dependent on the amplitude of stimulation and whether or not the subject is parkinsonian (Boulet et al., 2006).

**Activation of fiber tracts**

When stimulating a target, the focus of possible mechanism(s) usually lies within the stimulated targets and its neurons. The targets are small in size and usually surrounded by many tracts. The stimulation current can spread outside the borders of the anatomical target. STN is a small nucleus and it is surrounded by several major fiber tracts (Hamani et al., 2004). A computer
modelling study of STN stimulation in primates showed a significant activation of fiber tracts surrounding the STN (Miocinovic et al., 2006). In humans the finite element method (FEM) has been used in order to develop computer models of DBS electrodes and to create simulations of the electric field surrounding the electrode. The technique of modelling is now patient and treatment specific and can visualize the theoretical volume of probably activated tissue and tracts around the STN (Hemm and Wårdell, 2010). The tremor effect of STN stimulation has been hypothesized to be a result from direct activation of cerebello-thalamic fibers passing through the fields of Forel (Herzog et al., 2007). Microdialysis studies in animals receiving STN stimulation have shown significant increase in dopamine during stimulation, suggesting activation of the important nigro-striatal tract (Bruet et al., 2003, Lee et al., 2006, Meissner et al., 2002, Lacombe et al., 2007). The nigro-pallidal tract described both in animals and humans could also be activated especially in the early stages of PD, compensating for a loss of dopamine in the nigro-striatal pathway, leading to an enhancement of dopamine turnover in the GPi (Whone et al., 2003). In humans there have been no microdialysis studies performed to investigate any in vivo alterations of dopamine in the putamen due to activation of the nigro-striatal tracts. There exists some PET studies to measure dopamine binding in the striatum but they have failed to show changes during STN stimulation suggesting that in humans the therapeutic effects of STN stimulation is not mediated by striatal dopamine release (Hilker et al., 2003). The failure with PET studies to show a striatal dopamine release due to STN stimulation can be due to the fact that in the later stages of the disease there is not enough dopaminergic cells left in the SNc.
Regularization of pathological activity in target and neural network

It has been shown that HFS with frequencies above 100 Hz provide symptom relief. Previous studies have shown that HFS replaces pathologic irregular pattern with one that is time locked to the stimulus giving a more regular effect on downstream nuclei (Garcia et al., 2005). Neurochemical studies support this claim showing that low frequency stimulation don’t give the same neurochemical changes seen with HFS (Windels et al., 2003). Experimental data has shown that neural pattern, rather than firing rate, is an important determinant of the pathologic state and therapeutic effects seen with DBS (Hashimoto et al., 2003, Vitek 2002). Other experimental studies suggest that STN stimulation decreases neuronal burst activity in the STN and its target nucleus, the GPi, and as a result a reduction of pathological activity and its transmission through the network could be responsible for amelioration of motor symptoms during DBS (Meissner et al., 2005, Hashimoto et al., 2003, Shi et al., 2006). The beneficial effects of DBS can in part be due to modulation of the network activity which may not necessarily be restored to a pre-pathological state but rather to a third state that allows improved patient functioning (McIntyre and Hanh, 2010). This hypothesis of resetting oscillatory patterns is usually referred to as “jamming” of neural activity. A PET study has also indicated that suppression of network activity is a feature of both STN stimulation and lesioning (Trost et al., 2006).
The basal ganglia

General organization

The BG are a group of interconnected subcortical nuclei deep in the human brain hemispheres. The BG play a major role in normal voluntary movements including the initiation, regulation and termination of body movement. The BG are also involved in cognitive function and emotional behaviour (Chakravarthy et al., 2010).

The BG consist of several extensively inter-connected nuclei; the caudate nucleus, putamen, GPi, GPe, STN, and the two parts of the substantia nigra (SNC and SNr), see figure 5. The term striatum in the literature refers to the caudate nucleus and the putamen and sometimes the term lentiform nucleus is used to describe putamen and globus pallidum (Heimer, 1995).

The structures receiving most of the input to the BG is the striatum but the STN can also be considered as an input nucleus while it receives significant direct input from the cerebral cortex. The two main output nuclei of the BG are the GPi and the SNr. They innervate three known structures, the ventral anterior and the ventral lateral (VA/VL) nuclei of the thalamus, the superior colliculus and the pedunculo-pontine nucleus (PPN).

Through the VA/VL nuclei of the thalamus, the BG influence motor, sensory and cognitive cortical information processing. Through the PPN the BG influence spinal cord processing and aspects of locomotion and postural control. In contrast to the small number of output nuclei of the BG, the input arises from most of the cerebral cortex. Due to this the BG can influence many neuronal pathways and information processing systems (Utter and Basso, 2008, Smith and Kaplitt, 1998).
Figure 5. The diagram illustrates the current functional organization of the BG including the main neurotransmitters in normal state. GABA is inhibitory, Glutamate excitatory and Dopamine can be both inhibitory and excitatory depending on the type of dopamine receptor. Dopamine released in the striatum modulates corticostrial transmission and the Dopaminergic effect in the GPi is described as modulatory.

**Locomotion and BG**

Locomotion results from different complex neuronal circuits involving many areas in the brain. There are two described pathways for signal transmission through the BG, a direct and an indirect pathway.

In the normal state there is usually a balance between the two systems. In both the direct and indirect pathways the putamen and caudate nuclei are the first synapses in the system.
In normal state the direct pathway send activating signals from the motor areas of the cerebral cortex to the putamen and caudate nuclei, this activates the inhibitory projection neurons and increases the inhibitory output via the striatopallidal pathway to the GPi, resulting in a decrease of the tonic inhibition of the GPi’s output to the VA/VL complex of the thalamus. The pathways between GPi and the VA/VL nuclei are the lenticular fasciculus that passes through the internal capsule while the other pathway, the ansa lenticularis passes ventral to the internal capsule. The VA/VL nuclei send excitatory signals back to the cortical motor areas. In summary, the direct pathway results in a facilitation of the cerebral motor areas, which increase the ease of movement and of initiating movement.

The indirect pathway suppresses movements by increasing the inhibitory pathway by sending signals from the motor areas to the striatum, this facilitates the inhibitory projection neurons in the striatum that project to the GPe. In the GPe the tonic inhibitory output neurons are inhibited, resulting in reduced activity of the GPe. The decreased activity in the GPe results in decreased tonic inhibition of the STN, allowing more activation of the STN which in turn results in increased excitatory output from the STN to the GPi. The increased inhibition of the VA/VL nuclei decreases its output to the cerebral motor areas resulting in lesser activity (Belujon and Grace, 2011).

**Main neurotransmitters in the BG**

There are many neural pathways in the BG and they are either excitatory or inhibitory, depending on the neurotransmitters that are involved, see figure 5. Excitatory neurotransmitters are mainly glutamate while inhibitory neurotransmitters include GABA. Dopamine which is the main neurotransmitter in the important nigrostriatal pathway can be both inhibitory
and excitatory depending on the type of receptor they bind to in the striatum. There is also a dopaminergic innervation of the pallidum by a separate nigropallidal tract and/or by collaterals from the nigrostriatal tract (Jan et al., 2000, Chen et al., 2011). There are five different subtypes of dopamine receptors: D1, D2, D3, D4 and D5. The five receptors are individually categorized into two groups based on their varying properties and effects, the D1-like and D2-like subfamilies. The D1-like receptors have various effects on neuronal activity (excitatory), while the D2-like receptors tend to decrease action potential generation and are therefore usually considered inhibitory (Siegel 2006). Enkephaline and substance P are peptides that can act as neurotransmitters/neuromodulators and these are also found in the BG (Utter and Basso, 2006). Serotonin (5-HT) is a neurotransmitter released from cell bodies in the raphe nucleus and is widely spread in the basal ganglia through a complex distributional pattern (Parent et al., 2011). There exists a functional interaction between 5-HT and the dopaminergic system. It has been shown that 5-HT axons arborize densely and widely as the dopamine axons at striatal level. A result of the 5-HT/dopamine interaction is the capability of 5-HT terminals to convert exogenous L-dopa to dopamine. Dopamine can be stored and released at the 5-HT terminals through the vesical monoamine transporter-2 (Di Matteo et al., 2008). This can have two effects in PD, one is that 5-HT terminals can act as a local source of dopamine, on the other hand the striatal 5HT terminals cannot properly control the release of dopamine which can lead to an excessive non-physiological stimulation of dopamine receptors. This can play part in the development of LID which is a major side effect during treatment of PD (Carta et al., 2007, Parent et al., 2011).
Introduction

Nucleus Subthalamicus

The STN is regarded as an important structure for the modulation of activity of output in BG structures, especially the GPi. It is thought to play a prominent role in the pathophysiology of PD. It is the largest nucleus in the subthalamic area. The subthalamic area consists of the STN, thalamic reticular nucleus, zona incerta (Zi) and the fields of Forel.

The STN is a biconvex-shaped nucleus surrounded by dense myelinated fibers. Its anterior and lateral limits are enveloped by fibers of the internal capsule that separate the STN laterally from the GPi. Postero-medially it is adjacent to the red nucleus. Rostro-medially the STN abuts on the nucleus of the fields of Forel, the field H of Forel. The ventral limits of the STN are the cerebral peduncle and the SN (ventrolaterally). Dorsally the STN is limited by a portion of the fasciculus lenticularis and the Zi (Hamani et al., 2004, Schaltenbrand and Wahren 1977).

There are a number of fiber tracts coursing near the border of STN and some of the interesting tracts are the subthalamic fasciculus that consists of fibers that interconnect the STN and the GPi. The ansa lenticularis contains fibers from the GPi that projects to the thalamus and the fibers course posterior to enter the H Field of Forel. The lenticular fasciculus contains pallido-thalamic fibers and is designated H2 Field of Forel.
Figure 6. Representation of the major anatomical structures and fiber tracts associated with the subthalamic nucleus. AL=ansa lenticularis; CP=cerebral peduncle; FF = Fields of Forel; GPe =globus pallidus externus; GPI = globus pallidus internus; H1 = H1Field of Forel (thalamic fasciculus); IC = internal capsule; LF =lenticular fasciculus (H2); PPN = pedunculopontine nucleus; Put =putamen; SN = substantia nigra; STN = subthalamic nucleus; Thal= thalamus; ZI = zona incerta. Hamani et al. Brain (2004) Vol. 127 No1: 4. Courtesy of Oxford University Press, licence number: 3022430145305.

Nigrostriatal dopaminergic fibers leave the SNc and course just medially and dorsally to the STN (Hamani et al., 2004). The average number of neurons in each STN varies between different species and has been estimated to 560000 in humans (Hardman et al., 2002).

The STN has in primates several distinct subdivisions including motor, associative and limbic parts (Joel and Weiner, 1997). There are a number of afferent projections to the STN including cortico-subthalam, pallido-
subthalamic, thalamo-subthalamic and brainstem tracts, see figure 7. Efferent projections include, in PD, the important subthalamo-pallidal pathway, the subthalamo-nigral projections to the SNr and in rodents and non-human primates to both SNr and SNC. Other efferent projections include the pedunculopontine nucleus, PPN (Hamani et al., 2004). The pallido-
subthalamic tract connecting the GPe and STN is inhibitory using GABA as neurotransmitter while the efferent subthalamo-pallidal (GPi) tract is excitatory using glutamate as neurotransmitter.

**Figure 7.** Representation of the major subdivisions of the STN and its afferent and efferent connections. The STN has a volume of approximately 240 mm$^3$ and measures approximately 8 x 6 x 5 mm (Hardman et al., 2002).
Microdialysis

General considerations
Microdialysis is a well-established technique for continuous sampling of small water-soluble molecules within the extracellular fluid space in vivo (Chefer et al., 2009). The first papers on membrane based in vivo sampling of interstitial compounds were published already in 1966 by Bito who described the possibility of using a semi-permeable membrane to sample free amino acids and other electrolytes in the extracellular fluid of the brain and blood plasma of the dog (Bito et al., 1966). This study was followed by a paper from Delgado in 1972 and in 1974 Ungerstedt and Pycock presented the first attempt to use a membrane similar to the one we use today for microdialysis (Ungerstedt and Pycock, 1974). In 2012 approximately 14500 scientific papers have been published using this technique, and among them 2000 clinical investigations. The basic principle of microdialysis is primarily explained by Fick’s law of diffusion, which results in the passive diffusion of molecules across a concentration gradient. The microdialysis probe, consisting of a semipermeable membrane is continuously perfused with a perfusate that resembles the interstitial fluid. The perfusate equilibrates with the surrounding tissue fluid due to bidirectional diffusion. The concentration gradients of the interstitial fluid and the perfusate are the driving forces in this process. Microdialysis is a complex interplay between the dialysis membrane, the perfusate, the tissue and the extracellular fluid containing the molecules of interest, see figure 8. Microdialysis can be used both for collecting a substance as well as delivering it into the tissue (retrodialysis).
The microdialysate is collected at the end of the outlet tubing in vials suitable for small volumes. The substances being sampled are limited by the pore size of the microdialysis membrane, named cut-off. In our studies we used membranes with a cut-off of 20 kDa, which is suitable for L-dopa, one of the substances studied, with the molecular size of 197.2 Da. Today a wide range of microdialysis membranes are available enabling the sampling of molecules in sizes ranging from a few hundred Daltons up to 100 kDaltons. This allows sampling of molecules of greater molecular weight and it has extended the investigations to include inflammatory mediators such as cytokines.

**Figure 8.** The microdialysis catheter mimics a blood capillary. Substances from the extracellular fluid of the tissue diffuse across the membrane of the catheter into the perfusion fluid inside the catheter. The perfusate may flow either from the inner tube and out or in the opposite direction. Courtesy of CMA Microdialys, Sweden.

Microdialysis is very suitable for monitoring energy metabolites, neurotransmission, amino acids, and concentrations of certain drugs in target
tissues. One of the main advantages of *in vivo* brain microdialysis is that it enables studies of local brain regulation of pathophysiological processes in neurodegenerative disorders like Parkinson’s disease.

**Recovery – relative and absolute**

The dialysing properties of the microdialysis probe describes the ratio between the concentrations of a substance in the dialysate to that in the periprobe fluid, this is called relative recovery. Relative recovery will approach 100% as the flow rate approaches zero and decreases as the flow rate increases. The relative recovery is dependent on different factors (Plock and Kloft, 2005):

1. velocity of the diffusion process across the membrane which depends on (A) temperature (B) weight cut off and membrane area (C) concentration gradient
2. composition of perfusate
3. flow rate
4. tortuosity of the sample matrix

Absolute recovery is defined as the mass of a substance recovered during a defined time period. It is zero when the flow rate is zero and will reach a maximum at higher flow rates. When the concentration of a substance outside the probe changes, the concentration gradient across the membrane changes correspondingly. This results in an unchanged relative recovery but an increased absolute recovery.
Relative recovery will be regarded as constant as long as the conditions of diffusion are similar, while the absolute recovery varies with the interstitial concentration of the studied substance.

**Safety and limitations of microdialysis**

Microdialysis is an invasive technique used both in research and in clinical practice. From our experience with microdialysis in neurointensive care we know that the possibility to cause injury due to the catheter insertion is minimal. A limitation of the technique is the time resolution; mean values for a defined period are given rather than real-time data. Determination of the recovery may be time-consuming and require additional experiments. The recovery is largely dependent on the flow rate: the lower the flow rate, the higher the recovery. In clinical or research practice the flow rate cannot be decreased too much since either the sample volume obtained for analysis will be insufficient or the temporal resolution of the experiment will be lost. It is therefore important to optimize the relationship between flow rate and the sensitivity of the analytical assay. Previous studies have also shown that microdialysis in the brain may not be suitable for long term studies since the membrane may be clogged and gliosis in the surrounding tissue may occur (Georgieva et al., 1993). The formation of fibrin deposits that can clog the membrane can be inhibited by adding sodium dalteparin in the dialysis solution (Dizdar et al., 1999).
AIMS OF THE THESIS

Study I-IV:

I. The aim of this study was to investigate how much of the L-dopa in blood crosses over the blood brain barrier and the effects of the enzyme inhibitors entacapone and carbidopa on the L-dopa concentrations in blood and CSF.

II. The aim of this perioperative study was to develop a useful stereotactic microdialysis method for the study of neurotransmitter alterations during DBS and for the pharmacokinetics of L-dopa in brain tissue.

III. The overall aim was to develop a FEM model for prediction of the tissue volume from which biochemical data is obtained. A second aim was to implement the model with pre- and post-operative images for patients undergoing microdialysis in parallel to DBS, in order to structure-specifically predict the location and associated sampling volume of each microdialysis catheter.

IV. The aim of this study is to continue the work with accessing L-dopa and other neurotransmitters in the brain in combination with DBS treatment. Can alterations in neurotransmitter levels be related to the indirect pathway of locomotion? A second aim is to evaluate if there is any interference between L-dopa and DBS.
MATERIAL AND METHODS

Patient selection
The patients, in paper I, suffered from Parkinson’s disease with wearing off symptoms and where treatment with enzyme inhibitors could benefit the patients. They were sampled from the outpatient clinic. The patients gave written informed consent for participation in the study (ethical approval No. 20020115). The patients participating in study II-IV had advanced Parkinson’s disease and were referred for DBS therapy. The patients received thoroughly oral information and written informed consent was obtained (ethical approval No. 51-04). The patients in study III and IV were the same except for an additional patient in study IV.

Calf brain
In paper III an *ex vivo* experiment was performed with retrodialysis in basal ganglia from calf brain obtained from the local slaughterhouse. The use was approved by the Swedish Board of Agriculture, D.O. 38-172/09.

Stereotaxy and Planning
Leksell stereotactic system (model G, Elekta instrument AB, Sweden) was used in all stereotactic procedures. It is a long time used system with high precision, ≈ 1-2 mm. Leksell® Surgiplan System (Elekta Instruments AB, Sweden) was used for stereotactic calculation of targets and trajectories.

DBS equipment
The DBS system used in study II-IV was purchased from Medtronic (Medtronic Inc. USA). An Activa® PC 37601 or Kineta® 7428
neurostimulator, DBS extension cables Model 37086/7483 were used in combination with brain electrode Model 3389.

![Geometrical dimensions of the Medtronics 3389 quadripolar brain electrode. Each contact is 1.5 mm long and separated by a 0.5 mm spacing.](image)

**Figure 9.** Geometrical dimensions of the Medtronics 3389 quadripolar brain electrode. Each contact is 1.5 mm long and separated by a 0.5 mm spacing.

**Surgical procedure**

The stereotactic surgical procedures with implantation of the DBS system and microdialysis catheters were performed in the same manner for patients involved in study II-IV except for that the patients involved in study II had their surgical procedures performed in local anaesthesia with peroperative macrostimulation and subsequent neurological examination by the attending neurologist. We experienced from the procedures that we very seldom had to change the electrode position and for the convenience of the patient we performed the surgical procedure in paper IV in general anaesthesia.

The same neurosurgeons performed the procedures. The procedure starts with the placement of the Leksell® Stereotactic Frame model G (Elekta Instrument AB, Sweden). Direct anatomical targeting (Hariz et al., 2003) was performed in the STN and GPi on stereotactic MRI studies performed with a 1.5 tesla scanner (Achieva, Philips Healthcare, The Netherlands). Contiguous transaxial slices of 2 mm thickness, T2-weighted sequences for STN and Putamen and proton density and T1-weighted sequences for the GPi were collected together with coronal sequences. The stereotactic images were
exported to Leksell® Surgiplan System (Elekta Instruments AB, Sweden) for calculation of trajectories and targets. The GPi was chosen 2 mm anterior to the midcommisural point, 2-3 mm lateral of the pallidocapsular border on the axial slices and just above the optical tract on the coronal slices. The STN was visually chosen at the line connecting the anterior borders of the red nucleus at the level of their maximal diameter and approximately 1.5 mm lateral to the medial border of the STN. At surgery two standard burr holes were drilled on the coronal suture bilaterally, approximately 3 cm from the midline, for the placement of the DBS electrodes. Adjacent anteriorly to the right burr hole a 5 mm burr hole was drilled for the microdialysis catheter in study II and bilaterally in study IV. After the burr holes were drilled, the microdialysis catheters were inserted. Fluoroscopy images were captured during insertion of the DBS electrode and microdialysis catheter. During insertion, the catheter itself was not visible, only the catheter insertion needle. The gold tip of the catheter was not visible on fluoroscopy. The catheters were tunnelated out through a posterior skin incision and to fixate the catheters in the burr hole we used soft bone wax or fibrin glue (study IV). During the tunnelating procedure the catheters had to be held in place gently, otherwise they could easily dislocate. After DBS electrode and catheter placement the extension cable and the neurostimulator were implanted. The patients in study II where microdialysis was performed perioperatively had their catheters removed after the sampling was over. The patients in study IV had their microdialysis catheters for approximately 72h after which the catheters were removed. A postoperative CT scan without stereotactic frame was done in all patients after the implantations for visualizations and simulations of the microdialysis
catheters. The postoperative scan was also used in Leksell® Surgiplan System for image fusion with preoperative MRI to confirm electrode position.

<table>
<thead>
<tr>
<th>Anatomical target</th>
<th>Stereotactic coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>STN</td>
<td>12 mm lateral</td>
</tr>
<tr>
<td></td>
<td>2-4 mm posterior</td>
</tr>
<tr>
<td></td>
<td>3 mm inferior</td>
</tr>
<tr>
<td>Gpi</td>
<td>20-22 mm lateral</td>
</tr>
<tr>
<td></td>
<td>2-3 mm anterior</td>
</tr>
<tr>
<td></td>
<td>3-6 mm inferior</td>
</tr>
</tbody>
</table>

Table 1. Typical stereotactic target coordinates in relation to the midcommisural point that were compared to targets coordinates received at direct MRI targeting.

**Microdialysis**

This technique was used or described in all papers, I-IV. The catheters used for microdialysis sampling in the peripheral venous system in paper I, II and IV (CMA 64, CMA Microdialysis AB) are commercially available and the brain catheters in paper II-IV are custom made and CE marked for stereotactic use (CMA 65, CMA Microdialysis AB), see figure 10. These catheters have an extra long shaft, 19 cm that makes it suitable for stereotactic use. The catheters were manufactured with a small gold tip at the end of the membrane to make the catheter position visible with radiologic techniques.

It has been shown in a previous study that the recovery for catecholamines are better with the membrane cut off 20 kDa compared to 100 kDa, 64% and 13% respectively. The *in vitro* recovery for L-dopa is higher in ringer acetate at pH 4.0 compared to pH 7.0 (Blomquist et al., 1991). It is of importance that chatecolamines are collected in an acidic environment for preventing auto oxidation of the samples. In these studies ringer-acetate was used as perfusion fluid. After every patient an *in vitro* experiment was performed to calculate the relative recovery for the individual catheters. Ringer-acetate with known
concentrations of L-dopa was used in all recovery experiments. The recovery values for the brain catheters were between 40-90%. Fragmin 20 IU/mL was added into the perfusion for preventing fibrin aggregation on the dialysis membrane as described in a previous paper (Dizdar et al., 1999). The samples were regarded as protein free. In all collecting vials HCl was added to prevent auto-oxidation.

In our studies we found that a flow rate of 0.5 µL/min was optimal in combination with a 10 mm membrane. Due to the length of the catheters, the time lag of the catheter was calculated to 7.5 min. This means that the first fraction after each change only partly reflects the induced changes in the surrounding tissue due to the delay in transport.

Figure 10. Shows the different parts of the microdialysis system and comparison of the CMA 64 and CMA 65 shafts; (1) Perfusion pump with dialysate (2) Collecting vials (3) 19 cm long catheters for stereotactic use, CMA 65 (4) semipermeable membrane prelabelled with a gold tip (5) the CMA 64 catheter used for continuous sampling in the peripheral venous system. The catheters used in article II and IV were custom maid by CMA Microdialysis AB and CE marked.
Material and Methods

Figure 11. Geometrical dimensions of the CMA 65 microdialysis membrane.

High-performance liquid chromatography

High-performance liquid chromatography (HPLC) is a chromatographic technique that can separate a mixture of compounds and is used in biochemistry to identify, quantify and purify the individual components of the mixture. HPLC typically utilizes different types of stationary phases, a pump that moves the mobile phase and analyte through the column, and a detector to provide a characteristic retention time for the analyte. The detector may also provide additional information related to the analyte. Analyte retention time varies depending on the strength of its interactions with the stationary phase, the ratio/composition of solvent used, and the flow rate of the mobile phase. It is a form of liquid chromatography that utilizes smaller column size, smaller media inside the column, and higher mobile phase pressures. With HPLC, a pump provides the higher pressure required to move the mobile phase and analyte through the densely packed column. The increased density arises from smaller particle sizes. This allows for a better separation on columns of shorter length when compared to ordinary column chromatography. The peak height of the analyte must be clearly separated from the baseline. The HPLC analysis in study I and II were performed at the Department of Clinical Chemistry by laboratory research technicians and the HPLC analysis of the microdialysis samples in study IV were purchased from Pronexus Analytical AB, Stockholm, Sweden.
Computational modelling related to DBS

The difficulties and limitations of investigating and evaluating DBS and brain microdialysis in vivo are extensive. In order to overcome some of the practical difficulties and aid experimental research into how DBS work, how currents are spread around the electrodes, the Finite element method (FEM) technique has emerged (Åström et al., 2009). FEM is a way of describing physical parameters in a defined system by mathematical equations. The geometry, material properties and boundary conditions of the system are specified, together with a governing equation (depending on the application of interest), and the distribution of the parameters of interest throughout the system can then be calculated. The FEM technique was used in paper III by the first author.

Figure 12. Axial MRI slice of the basal ganglia illustrating an example of computational modelling and simulation with FEM of electric field around the STN electrodes (green) and simulations of the sampling area (TVImax) around the microdialysis catheters (orange) located in the GPi. The microdialysis catheters are positioned in the GPi bilaterally and in the right putamen. Commercial FEM software (Comsol Multiphysics 3.5, Comsol AB, Sweden) was used to simulate analyte diffusion and electric currents in the brain.
Statistics

Paper I: The statistical methods and calculations were done with the help of statisticians. ANOVA, with repeated measures, was used to calculate differences of the maximum concentration (C_{max}) values between the days. Adjustments were made with regard to the individual and time of the measurement. The area under the concentration time-curve (AUC) was calculated by linear trapezoidal summation to time. 95% confidential interval (CI) was calculated for AUC. P < 0.05 was regarded as significant.

Paper II: This paper is to be considered as developing a methodology for stereotactic microdialysis. The results are regarded more descriptive and presented without any specific statistical evaluation except for mean values.

Paper III: This paper contains complex mathematical and statistical measurements for describing the simulation and visualization processes. The statistical and mathematical calculations were conducted by the first author E. Diczfalusy.

Paper IV: This is a descriptive paper. Some results for the different substances measured are shown as mean values. The microdialysis results are primarily used as a qualitative measurement of potential changes in neuronal activity.
REVIEW OF THE PAPERS AND THE MAIN RESULTS

Paper I: “The effect of peripheral enzyme inhibitors on levodopa concentrations in blood and CSF”

Paper II: “Stereotactic microdialysis of the basal ganglia in Parkinson’s disease”


Paper IV: “Neurotransmitter levels in basal ganglia during levodopa and DBS treatment in Parkinson’s disease”
Paper I (published in Movement Disorders 2011)
The Effect of Peripheral Enzyme Inhibitors on Levodopa Concentrations in Blood and CSF

Aim: This paper is the first in the thesis and the aim of this study was to investigate how much of the L-dopa in blood crosses over the blood-brain-barrier and the effects of the enzyme inhibitors entacapone and carbidopa on the L-dopa concentrations in blood and CSF.

It has been shown that $C_{\text{max}}$ can be an important factor in the development of drug induced dyskinesias. It is believed that alterations in $C_{\text{max}}$ can give pulsatile stimulation of receptors which can induce alterations in the postsynaptic dopamine receptors and that this can be a cause of dyskinesias. Another aim was to investigate if the enzyme inhibitors increase the Area Under Curve (AUC), see figure 13, without increasing the $C_{\text{max}}$.

Figure 13. The figure illustrates a possible entacapone effect with increase in AUC and Cmax.
**Method:** Five PD patients with L-dopa/benserazide treatment and experience of “wearing off” phenomenon underwent microdialysis in blood and sampling of CSF during the following treatments:

**Day 1:** i.v. L-dopa infusion (30 mg/h) during 12h.

**Day 2:** i.v. L-dopa infusion (30 mg/h) during 12h with additional oral entacapone, 200 mgx3.

**Day 3:** i.v. L-dopa infusion (30 mg/h) during 12h with additional oral entacapone, 200 mgx3 and oral carbidopa, 25 mgx2.

The patients received a microdialysis catheter in a brachial vein. We used microdialysis because it is a continuous monitoring and because it allows us to draw only small amounts of dialysate frequently compared to the option of taking blood samples each 30 minutes during daytime for three days. From the lumbar drainage CSF samples were taken every two hours under sterile conditions.

**Results:** The study showed that when comparing the $C_{\text{max}}$ levels from the three study days the mean difference of the $C_{\text{max}}$ levels in CSF was 11% when adding the COMT inhibitor to levodopa and 121% when entacapone was combined with the AADC inhibitor carbidopa. This increase is seen both in the CSF and in the blood and is more evident when entacapone is combined with carbidopa.

The study showed that, after correction for recovery, the ratio of L-dopa in the CSF from blood is 43 % in basal values.
**Figure 14.** The figure illustrates the mean L-dopa concentrations in the CSF during i.v. L-dopa infusion which was given during day 1. On day 2 the L-dopa infusion was given concomitant with oral entacapone (A-C). On day 3 the L-dopa infusion was combined with oral entacapone (A-C) and carbidopa (B-C). The curve from the third study day did not reach its maximum at the end of the collecting period after 12h.

The purpose from this study in this thesis was to obtain results from the pharmacokinetics of L-dopa and also to estimate how large fraction of L-dopa crosses the BBB and to use these results in consecutive studies concerning interactions between L-dopa and DBS. A step towards this was to evaluate how the enzyme inhibitors affect the AUC and if this induces an increased $C_{\text{max}}$ in CSF. An increased $C_{\text{max}}$ may play an important role in developing LID that can be improved with DBS.
**Paper II** (published in J Neurosci Methods 2012)

**Stereotactic microdialysis in the basal ganglia in Parkinson’s disease**

**Aim:** Development of a perioperative surgical method for stereotactic microdialysis in human brain using a microdialysis catheter with a gold tip for fluoroscopic target confirmation. Can L-dopa given intravenously be measured in the GPi? Can neurotransmitters (catecholamines) be measured in the human parkinsonian brain *in vivo*? Are there any alterations in catecholamine concentrations due to DBS?

**Method:** 10 patients with advanced PD eligible for DBS took part in the study. One microdialysis catheter was inserted stereotactically in the right GPi and sampling was started. DBS electrodes were placed bilaterally. The sampling period started 45 min after catheter insertion according to the following schedule:

- 30 min baseline monitoring
- 30 min STN stimulation
- 30 min baseline monitoring
- 45 min L-dopa infusion

Ringer-acetate with low molecular heparin was used as perfusion fluid. Samples were collected each 15 minutes. During the procedure, the attending neurologist performed clinical evaluation.

**Results:** The stereotactic method is suitable for measuring L-dopa and other catecholamines in the brain with microdialysis. The gold tip of the microdialysis catheter could not be visualized with fluoroscopy. The sampling
periods were too short for showing any significant differences of the studied transmitters during DBS. After adjustment to recovery of the catheters we found that approximately 18% of the L-dopa concentration in blood reaches the brain. Eight of the patients who were on L-dopa medication preoperatively had unexpected high concentrations of L-dopa in Gpi, which could indicate a possible storage capacity of L-dopa in the brain without metabolising to dopamine. This would mean that L-dopa has a longer half-life in brain tissue than in blood ($T_{1/2} 30$ min) since the medication was discontinued at least 12 h prior to surgery. In early stages of the disease it has been suggested that L-dopa is metabolised to dopamine, which then is stored and released between oral intakes of L-dopa.

**Figure 15.** The figure illustrates L-dopa values adjusted for recovery in the right GPi and in the blood. (A) shows an increase and a subsequent decrease of L-dopa concentration in the GPi during STN stimulation, (B) shows the increase in the last three fractions during L-dopa infusion. The first sampling started 45 min after catheter insertion and each fraction was 15 min.

A model for simulation and patient-specific visualization of the tissue volume of influence during brain microdialysis

**Aim:** The overall aim was to develop a FEM model for prediction of the sample volume around the microdialysis catheter and to evaluate the model input parameters using statistical analysis and experimental data. To interpret biochemical data in relation to anatomical targets in the brain.

A second aim was to implement the model with pre- and post-operative images for patients undergoing microdialysis in parallel to DBS, in order to structure-specifically predict the location and associated sampling volume of each microdialysis catheter. In order to estimate diffusion in the deep brain structures, a microdialysis experiment was performed on brain tissue from calf.

**Method:** Four patients (age 56 ± 8) with advanced PD took part in the study. During surgery for DBS microdialysis catheters were stereotactically inserted in the right Putamen and in the GPi bilaterally. The CMA 65 catheter with a gold tip was used. The patients underwent continuous microdialysis according to a specific schedule for 72h postoperatively. Postoperatively a stereotactic CT without frame was done in all 4 patients and these examinations were image fused with preoperative MRI using the Leksell® Surgiplan System (Elekta Instruments AB, Sweden). A microdialysis FEM model was developed for simulation and visualization of the catheters to investigate the anatomical position of the catheters and \( rTVI_{\text{MAX}} \) was calculated. The \( rTVI_{\text{MAX}} \) was superimposed on ex vivo retromicrodialysis studies on calf brain.
**Results:** In this paper the gold tip of the catheters visible on CT allowed us to show with high precision in which nuclei the catheters are located and that the membrane is within the intended target. It’s crucial for the anatomical interpretations and the FEM simulations to know exact were the catheter tip is located.

![CT scan illustration](image.png)

**Figure 16.** The image illustrates the postoperative CT scan of one of the patients in the postoperative study. (A) the gold tip of the putaminal catheter (B) and (C) shows the bilateral STN electrodes.

The study shows that analyte diffusion simulations for dopamine can be combined with post-operative patient images, in order to visualize the maximum tissue volume that is being sampled during brain microdialysis, it shows that measurement sites are correct and comparable between patients when interpreting physiological data.

The extension of the rTVI\(_{\text{max}}\), 0.85±0.25mm, is also reasonable in comparison to previous studies, predicting that the microdialysis sampling depth for similar substances would be within the millimetre range.
My interest was the interpretation of pre and postoperative radiology with the FEM simulations and the interpretation of the sampling volume simulated with calf brain experiment.

**Figure 17.** Axial MRI slice illustrating the boundaries of the basal ganglia nuclei with the microdialysis catheters (yellow) and STN electrodes (green).
Paper IV (submitted)

Neurotransmitter levels in basal ganglia during levodopa and Deep Brain Stimulation treatment in Parkinson’s Disease

Aim: The aim of this study was to measure neurotransmitters in patients with implanted DBS in STN and to see if there are any changes in neurotransmitter substances during stimulation on/off and during L-dopa infusion. A second aim was to see if there is any interaction between L-dopa therapy and DBS explaining the possibility to reduce L-dopa medication postoperatively.

Method: Five patients with advanced PD and implanted DBS took part in the study. Four of these patients were the same as in paper III. The microdialysis catheters were placed as described previously in paper III. The study lasted for 72h and the patients were monitored in the neurosurgical postoperative ward according to a specific study schedule. Stimulation parameters were the same as used in paper II: monopolar stimulation 2V, 130 Hz and 60 µs.

- baseline monitoring 14h (from end of surgery to the next morning)
- stimulation left side 3h
- baseline monitoring 1h
- stimulation right side 3h
- baseline monitoring 1h
- bilateral stimulation 3h
- baseline monitoring 14h
- L-dopa infusion 3h
- baseline monitoring 1h
- L-dopa infusion + bilateral stimulation 5h
- baseline 1h
continuous bilateral stimulation

**Results:** A large number of neurotransmitters and amino acids were analysed including L-dopa, dopamine, HVA, serotonin, glutamate and GABA.

In this study we could show that DBS STN has an effect on dopamine in several of the patients. During DBS we can see an increase of putaminal dopamine release indicating that STN might have a direct action on the SNc alternatively affecting the nigrostriatal tract that is in close relation to the STN and can be effected by the electrical field.

![Figure 18](image)

**Figure 18.** The figure illustrates the dopamine concentrations in one patient during (A) 3h left sided STN stimulation, (B) 3h right sided STN stimulation, (C) 3h of bilateral STN stimulation, (D) 3h of L-dopa infusion 75 mg/kg i.v., (E) 5h of concomitant L-dopa infusion and bilateral STN stimulation. After (E) the patient continued with bilateral STN stimulation.

During L-dopa infusion and concomitant DBS there was an increase in the GABA concentrations in the right Gpi compared to baseline values indicating an interaction between dopamine and GABA.
Figure 19. The figure illustrates GABA concentrations in the right GPi. (A) 3h of L-dopainfusion 75mg/kg i.v., (B) L-dopa infusion with concomitant STN stimulation during 5h, (C) during bilateral STN DBS. During bilateral DBS alone the GABA concentration is decreasing to baseline values. The graph represents the mean values of three patients.

Interestingly we found evidence of an interaction between L-dopa and DBS resulting in higher levels of L-dopa during concomitant treatment with L-dopa and DBS. This could in part explain why it is possible to reduce L-dopa medication after surgery.

<table>
<thead>
<tr>
<th></th>
<th>CSF/blood %</th>
<th>Gpi/CSF %</th>
<th>Put/CSF %</th>
<th>Gpi/blood %</th>
<th>Put/blood %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal value</td>
<td>43,3</td>
<td>23,3</td>
<td>13,3</td>
<td>10,1</td>
<td>5,8</td>
</tr>
<tr>
<td>L-dopa infusion 75mg/h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-dopa infusion 75mg/h + STN-stimulation</td>
<td>18,6</td>
<td>16,8</td>
<td>20,3</td>
<td>19,4</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Illustrates L-dopa concentrations. In basal conditions the concentration of L-dopa in CSF is 43% of the concentration in blood. The relation of L-dopa in the GPi and Putamen to CSF was 23 % and 13 % respectively. The grey field show increases of L-dopa in GPi and Putamen during L-dopa infusion, with a further increase with up to 15% when L-dopa infusion was combined with bilateral STN stimulation.
DISCUSSION

The thesis includes papers I-IV and the goals were to study the effects of L-dopa and DBS treatments on human brain in Parkinson’s disease. The research started in paper I with the study of L-dopa passage over the blood brain barrier and the influence of enzyme inhibitors on the concentration levels of L-dopa in CSF. Intermittent non-physiological high levels of L-dopa in the brain may induce dyskinesia seen in PD. Focus in paper II and IV were on L-dopa levels in basal ganglia and the mechanisms of DBS. The overall aim in paper II and IV was to develop a microdialysis method for patient specific biochemical monitoring during DBS. Paper III describes anatomical visualization of microdialysis catheters and simulation of the tissue volume, which is dialysed during microdialysis collection.

Microdialysis

The microdialysis technique has been used in all four papers and the catheters were placed in both blood and brain tissue. Microdialysis values can be presented with or without adjustment to recovery. As described earlier relative recovery depends on different factors such as; the velocity of the diffusion process; the composition of perfusate; the flow rate and the tortuosity of the sample matrix. In our experiments, we used a uniform approach regarding membrane length, cut off, perfusate and flow rate. The variation of the in vitro recoveries for the brain catheters used in study II and IV (42% and 90% respectively) were due to change of membrane manufacturing. The in vitro recoveries were done under the same controlled circumstances.
Location of the microdialysis catheters to the exact area of interest is one of the most important issues in microdialysis studies while the dialysate reflects the biochemistry of the target. The 10 mm long membranous area of the catheters used in paper II and IV, enables sampling of the targets we studied. If considering studying a smaller target, like the STN or the substantia nigra, the length of the membrane and the flow rate may need adjustments.

During implantation a cannulated needle was used and insertion of the needle was stopped approximately 10 mm before reaching the target leaving the distal membrane of the catheter free to enter the area of interest. From the work with visualization of the catheters in paper III we found that two catheters were deviating and this was not depending on the accuracy of the stereotactic method but rather due to technical difficulties with the soft flexible catheters. Due to this, postoperative visualization and confirmation of target can be considered as crucial, when interpreting the microdialysis data.

Estimation of sampling area

The maximum tissue volume of influence, TVI$_{\text{max}}$, was used in paper III to make a qualitative approximation of the maximum tissue volume that could be sampled around the catheters. Estimation of TVI$_{\text{max}}$ gave us an assumption of how much tissue was dialysed along the membrane length but the method did not provide exact knowledge since we did not have detailed knowledge about the interaction between the analyte, the surrounding tissue, membrane properties and flow rate, which all could affect the TVI$_{\text{max}}$. Estimation of TVI$_{\text{max}}$ is done by simulating the analyte migration in all directions from the microdialysis catheter, according to Fick’s law of diffusion. A quantitative estimation of TVI$_{\text{max}}$ is not conducted since this requires knowledge about the interaction between the analyte and the surrounding brain tissue volume.
which is not possible. The $rTVI_{max}$ corresponds to the simulated maximum analyte migration distance, and is closely related to the microdialysis penetration depth. The $rTVI_{max}$ was estimated to $0.85\pm0.25$ mm for dopamine.

In an attempt to relate the simulation results to experimental data in paper IV we performed an *ex vivo* retrodialysis experiment with crystal violet solution in calf brains, see figure 20. Crystal violet was chosen due to its molecular weight of 408 Da, which is near the molecular weight of dopamine. The same flow rate, 0.5 $\mu$L/min, was used in the retrodialysis as in the *in vivo* studies with an infusion time of 1h.

An *ex vivo* experiment does not take in account important factors such as blood flow which influences the diffusion rate and the analyte clearance. Our *ex vivo* experiments showed a very good conformity with the simulation thus showing that the prediction of the $rTVI_{max}$ was reasonable.

![Figure 20](image)

**Figure 20.** The figure illustrates the calf brain experiment with retrodialysis of crystal violet solution into the brain, superimposed with circles corresponding to the $rTVI_{MAX}$ of dopamine.

**Microdialysis compared to other techniques**

The two alternative techniques that would have been available for these studies are Positron Emission Tomography (PET) imaging and Magnetic resonance spectroscopy (MRS). MRS and PET have the advantage of not being
invasive methods compared to microdialysis. MRS has a rather poor spatial resolution in contrast to PET, which has rather good spatial resolution of a few millimetres that allows assessment of regionally different drug concentrations. Microdialysis sampling is restricted to a small area with a better temporal resolution than PET and MRS. Microdialysis enables measuring of substances continuously, which makes the technique useful in studies over a prolonged time period with frequent sampling. On the other hand one of the drawbacks with microdialysis is the inability to give a momentary picture.

In the future, MRS could possibly become suitable for neurotransmitter studies, though the implanted DBS hardware constitutes a difficulty. The low L-dopa and dopamine concentrations in the basal ganglia cannot be studied with MRS today as this requires higher concentration levels. Some studies concerning MRS and implanted stimulators suggest MRS to become a feasible method for evaluating neuronal function in PD (Llumiguano et al., 2008, Chernov et al., 2008). One disadvantage with PET is the short half-life of the isotopes used with the maximum possible imaging time of approx. 2 hours (Brunner and Lang, 2006).

**L-dopa, enzyme inhibitors and dyskinesia**

L-dopa is the most effective treatment of motor symptoms in PD. At some point, usually after 2-5 years, most patients treated with L-dopa develop wearing off phenomena and LID. The most common form of LID is the peak dose dyskinesia, occurring at the highest plasma concentrations of L-dopa. There are several hypotheses concerning the development of LID. The most accepted is that oral L-dopa treatment causes a non-physiological variability of dopamine concentrations in brain tissue with pulsatile stimulation of striatal dopaminergic receptors. This induces plasticity changes
of the receptors and this in turn triggers the development of LID, see figure 21.

Most PD patients referred for DBS treatment have an advanced stage of the disease and are suffering from LID. STN DBS reduces LID but the mechanism behind this is unclear. One explanation is that STN DBS enables the reduction of oral L-dopa dosages and thus improving LID. On the other hand, in patients where L-dopa therapy is not decreased, we still see a reduction of LID during DBS. Most probably this is due to several interfering factors.

The enzyme inhibitors entacapone and carbidopa are combined with L-dopa to increase the bioavailability of L-dopa. Since increase of $C_{\text{max}}$ is considered unfavourable due to its role in developing LID, one of the aims of paper I was to investigate if increased AUC in blood causes an increase of $C_{\text{max}}$ in CSF. Most previous papers dealing with this subject only measured $C_{\text{max}}$ in plasma and not in the central nervous system. Kaakkola et al. who performed a similar study, only analysed L-dopa concentrations in plasma and only after one dose of entacapone (Kaakkola 1994).

As expected, in blood, the AUC of L-dopa increased with up to 50% when L-dopa was combined with entacapone and carbidopa compared to when it was given alone. Interestingly, in CSF, this combination gave a significant increase of L-dopa $C_{\text{max}}$ with up to 121%. Thus the results from this study confirm that entacapone and carbidopa increases the bioavailability of L-dopa, however the rise of $C_{\text{max}}$ should be kept in mind when prescribing these drugs. The drugs are available in different dosages and to avoid accumulation of L-dopa and $C_{\text{max}}$ spikes, one should consider tailored prescriptions.
Figure 21. This figure illustrates the proposed pattern of LID development according to several studies (Carta et al., 2007, Cenci and Konradi, 2010, Parent et al., 2011). DBS may interfere in this network of pathological activity.

L-dopa and DBS in basal ganglia

In paper II we used in vivo microdialysis in the brain for the first time. Some of the difficulties in performing these kinds of studies were to find 10 patients who both accepted participation and fulfilled the criteria since at our Department we performed 6-8 STN operations per year. The time span for sampling the patients in paper II and IV was 5 years.

No earlier studies with in vivo microdialysis in GPi measuring L-dopa were reported. GPi is the main output nucleus from the STN, which is why this target was chosen, both in paper II and IV. We know that a turnover of dopamine in the GPi exists and that it is mediated through a nigropallidal tract or by collaterals from the nigrostriatal tract (Rajput et al., 2008, Jan et al., 2000). Whone et al. (2003) demonstrated data that indicates a compensatory
upregulation in the nigropallidal dopamine projections to the GPi in early PD. There is little information about L-dopa and dopamine function in the two pallidal segments. We did not know if there would be any alterations of L-dopa or other neurotransmitters due to DBS and if the different time intervals for stimulation, baseline and L-dopa infusion would be optimal for observing any changes. Paper II revealed measurable L-dopa levels in the GPi and increased pallidal levels during both DBS and L-dopa infusion, especially in the eight patients that preoperatively were on L-dopa medication. Two of the patients, who were not on previous L-dopa medication, did not show the same increase of L-dopa concentrations during infusion. One probable explanation to this might be that the patients with previous L-dopa medication had clinically relevant levels of enzyme inhibitors remaining in the tissues although medication was disrupted more than 12 hours previous to surgery. Another explanation could be that the two patients without previous medication had shorter duration of PD with less degeneration of the dopaminergic system and that compensatory mechanisms with uptake of L-dopa by adjacent systems not were developed.

We know from literature and experience that stereotaxy is a well-documented and precise method but we also wanted to show that the catheter was placed into the intended target, the GPi. The catheters were manufactured with a gold tip for visualization by fluoroscopy but this could not be realized and validation of catheter placement was not achieved in paper II. Neurophysiological measurements with microrecording technique could have been an option to confirm target site, but we do not use this method in clinical practice. There is also a discussion about using microrecording since some
studies have shown a small but increased risk for intraparenchymal haemorrhage (Zrinzo et al., 2011).

The time in the operation theatre was prolonged by the study with 2h 15min. The issues that had to be addressed for the following paper, III and IV were; longer sampling time, verifying the positions of the catheters, and estimation of the sampling tissue volume.

**Simulation and anatomical visualization**

It is of greatest importance to have an exact knowledge of catheter positions when sampling is performed in small specific areas of the brain and when the biochemical results are to be compared between different structures. As earlier mentioned, the risk of a misplaced microdialysis catheter could be greater compared to implantation of leads due to the thin, flexible dialysis membrane that can deviate and also due to the difficulty of proper fixation to avoid catheter movement.

![Image showing patient 2-5 with outlined boundaries of Putamen, GPe and GPi in the basal ganglia. In patient 5, the right intended GPi catheter is located in the putamen.](image)

**Figure 22.** Patient 2-5 illustrates outlined boundaries of Putamen, GPe and GPi in the basal ganglia. In patient 5, the right intended GPi catheter is located in the putamen.
The FEM simulations were combined with patient specific preoperative MRI and postoperative CT images in order to obtain the exact positions of the catheters in the basal ganglia. The gold tip of the microdialysis catheter turned out to be crucial in localization of the catheter tip on postoperative radiology images.

The computer modelling studies with FEM showed in paper III that the simulations could be combined with images from the patients making the interpretation of the microdialysis data patient specific. FEM was used previously in several studies to create simulations of the electric field surrounding a DBS electrode (Åström et al., 2009, Åström et al., 2010). These simulations provide important input to the knowledge of the functioning of DBS. Knowledge of how the distribution of the electric field around an electrode with set parameters could help the clinician in the understanding of stimulator induced side effects.

**Postoperative microdialysis recordings**

The microdialysis data from the five patients participating in study IV could shed some light on the mechanisms of DBS. We chose to insert one catheter in the putamen for investigating any dopamine release during DBS, which was previously shown from animal studies (Lacombe et al., 2007, Bruet et al., 2003).

The GPi was chosen due to being the main output structure of the BG, regulating thalamocortical activity. GABA, Glutamate and dopamine are neurotransmitters controlling and modulating the activity of GPi, see figure 23.
Figure 23. The figure illustrates the major neurotransmitter pathways in the basal ganglia network in Parkinson’s disease. Red indicates GABA, green Glutamate and grey Dopamine. The hypothesis in PD is that there is a dysfunctional high activity of the STN due to decreased tonic inhibition from the GPe. This STN overactivity results in overactivity of GPi, which is the main output nucleus from the BG. This results in increased inhibition of the motor thalamus leading to decreased activity of the thalamocortical loop. The GABA mediated inhibition of the GPi from the striatopallidal tract is also affected resulting in lesser inhibition of the GPi. The nigrostriatal pathway is dysfunctioning due to few dopaminergic cells in the SNC and degeneration of the nigrostriatal tract.

In 3 out of 4 patients analysed for dopamine levels we found a putaminal release of dopamine during STN stimulation, see figure 24. The mechanism behind this may be explained by a direct effect of the STN on SNC, activating remaining dopaminergic cells and resulting in an increased putaminal release through the nigrostriatal pathway. Alternatively STN stimulation could affect
the nigrostriatal tract that passes close to the STN and therefore could be affected by current spread. Interestingly in one patient where no increase of dopamine was seen, the improvement of rigidity and tremor was similar to remaining patients. One explanation could be an inhibition mediated through the striatopallidal tract could also have an effect on rigidity and tremor.

**Figure 24.** This figure illustrates the microdialysis results from patient 2 in figure 22. (A) STN stimulation left side, (B) STN stimulation right side, (C) bilateral STN stimulation, (D) L-dopa infusion, (E) L-dopa infusion with concomitant bilateral STN stimulation. The induced release of dopamine by DBS was short lasting and could be due to release of limited amounts of stored endogenous dopamine from presynaptic vesicles. Interestingly, after discontinuation of L-dopa infusion but with continuing bilateral DBS stimulation, the dopamine concentrations decreased to baseline levels. A high analyte clearance could also affect the measurements around the catheters.

One of the patients accidentally received repeated oral doses of L-dopa, which was reflected soon in the microdialysis data confirming the rapid absorption and distribution to the brain of L-dopa, see figure 25. The outline of the curve shows high peaks after tablet intake with deep throughs between
doses. This could indicate how oral treatment with tablet medication causing highly alternating concentrations of dopamine might accelerate neurodegeneration and cause LID. The outline also shows that repeated intake of L-dopa tablets increased the $C_{\text{max}}$ confirming the results from paper I.

Figure 25. The figure illustrates Dopamine levels in putamen, GPi right and GPi left viewing the rapid pharmacokinetics of L-dopa. The arrows indicate the times this patient accidentally received L-dopa tablets. (A) left sided STN stimulation, (B) right sided STN stimulation and (C) bilateral STN stimulation.

The analysis of GABA in the right GPi, see figure 19, showed an increase of concentration during both L-dopa infusion and during L-dopa infusion with concomitant DBS stimulation. This could be due to GABA release from presynaptic terminals from the striatopallidal tract. The increased GABA concentration in the GPi, inhibits the nucleus that most probably is overactive in PD. This in turn, reduces the inhibitory effect of GPi on the motor thalamus, resulting in a reactivation of the thalamocortical pathway, which facilitates movement.
During bilateral DBS, the GABA levels decreased to baseline values. The pattern was the same as for L-dopa concentrations during DBS, indicating that DBS on its own does not have an effect on GABA.

Glutamate, another neurotransmitter in the STN-GPi pathway, was recorded without showing any significant changes in concentration, see figure 26. However, microdialysis can only detect neurochemical changes in the synaptic cleft if they are sufficiently reflected into the extracellular space and small but relevant changes in the synaptic cleft might not be seen in this analysis. Glutamate has also a very efficient reuptake into nerve terminals that could result in smaller amounts in the extracellular space for analysis with this method.

![Figure 26](image)

**Figure 26.** The figure illustrates mean glutamate levels in the right GPi in 3 patients with high concentrations several hours after catheter insertion during night. No pattern, correlating to DBS stimulation or L-dopa infusion, was seen. (A) left sided STN stimulation, (B) right sided STN stimulation, (C) bilateral STN stimulation. The same pattern was seen in the left GPi.

A summary of our hypothesis from the microdialysis data collected in paper IV are shown in figure 27.
Figure 27. The figure illustrates our hypothesis built on the microdialysis data collected from the patients in study IV. The following possible mechanism(s) are proposed; increased dopaminergic putaminal release due to a direct effect of STN on the SNc and an increased inhibition of the GPi, due to increased activity of the striatopallidal tract, leading to lesser GPi activity on motor thalamus. This could facilitate the thalamocortical loop enhancing movement.

We compared all basal endogenous levels of L-dopa from blood, CSF and brain tissue from paper I, II, and IV to study the relations of the concentration values between these stuctures. All results were corrected for recovery. We found the levels to be; in CSF 43%, in GPi 10% and in Putamen 6% of the levels found in blood. In paper II during L-dopa infusion we showed that 18% of L-dopa crosses the BBB to GPi. When we compared the values from the patients in paper IV we found the same figures and the total mean value is 18% for all
patients included in paper II and IV. When adding DBS to ongoing L-dopa infusion, in paper IV, we could observe a further increase of L-dopa in the BG by 15% as shown in table 2. This pattern was seen in all 5 patients and could partially explain why it is possible to reduce L-dopa medication after DBS surgery with up to 50%. Taken into account that this is seen almost immediately after surgery, it could be possible that this effect could be more pronounced over time if STN DBS induces long-term plastic changes of the dopaminergic system.

Ethical permission for the postoperative study in paper IV was given for 15 patients. The first aim was to set up logistics and evaluate results. After 5 patients the microdialysis samples were analysed and presented. We decided to publish this data before continuing with further more patient specific postoperative studies.

This is the first time we can demonstrate a postoperative validation of the microdialysis catheter positions and also with an estimation of the sampling area around the catheters. In previous microdialysis studies this was not done (Fedele et al., 2001, Galati S et al., 2006, Kilpatrick et al., 2010, Stefani et al., 2005, 2006, 2011). PD is often reported as a relatively homogenous disease but the data from this study, indicates that large individual variations could explain the differences of the obtained data.

The relative long sampling times in study IV (1h) does not allow detection of rapid changes in neurotransmitters.

**Ethical aspects**

In studies where invasive methods are used on humans (article I, II and IV) we have put a lot effort into informing the patients about the risks of participating in the studies. We could not see any side effects caused to the patients due to
their participation in the procedures. The most serious complication would have been an intracerebral haemorrhage due to the placement of the brain microdialysis catheters. Meticulous preoperative neuroimaging in planning the trajectory and for target verification decreases the risks. Elderly patients with hypertension were not included in the studies.

At the time of the studies we had experience from performing approximately 120 DBS procedures involving some 180 lead trajectories. In one case we had a small asymptomatic intraparenchymal haemorrhage. The risk of haemorrhage in our material (1/180) is 0.0055 %. In future studies one should consider using fewer catheters to further minimize the risks of adverse events for the patients.

A difficulty with studies were microdialysis in the brain is performed is the few patients enrolled, in previous studies published, the number of patients vary between 5-15 and even though the theory behind the research can be explained by the results observed, the generalizibility is low.

All of the patients enrolled in the studies had excellent clinical results of the surgical treatment.

**Future approaches**

During the last 20 years there have been many experimental approaches to studying the mechanisms of LID and DBS and some of them have been mentioned in this thesis. There are many methodological differences in all approaches to the subject of underlying mechanism(s) in DBS that can affect the responses and are important to consider.

Using microdialysis *in vivo* on humans is a safe method for the study of local neurochemical changes and interactions during DBS. It may also be a good method for further studies of pharmacologically active drugs, like L-
dopa in brain tissue. Continued work with simulations of electrical fields surrounding electrodes can also provide important input to the mechanism(s) of DBS. The mechanisms of LID and DBS are most probably very complex involving many neural structures in the BG circuitry and these studies hopefully can shed some light on this issue. These studies have shown us that it is possible to measure neurotransmitters and L-dopa, with high accuracy in the basal ganglia in combination with DBS.

Future studies on DBS mechanisms should be more patient specific with different stimulation settings and longer stimulation times. A more patient specific approach could help us to find the best possible stimulation parameters resulting in an individual biochemical optimum for the patients studied. More concern could also be taken when sampling the patients, to obtain a more homogenous study group.

This research, especially paper III and IV, was performed with an interdisciplinary team consisting of neurosurgeon, neurologist, engineers and biochemists.
Biokemiska studier vid Parkinson’s sjukdom

Parkinson’s sjukdom kännetecknas av en tilltagande brist på signalsubstansen dopamin i hjärnan. Bristen på dopamin leder till en rad motoriska symptom hos patienterna; skakningar, stelhet och minskade rörelser vilket leder till sämre livskvalitet. Under 1960-talet startades behandling med L-dopa som i hjärnan omvandlas till dopamin. Sedermera har det tillkommit nya läkemedel, sk enzymhämmare som hjälper till att bibehålla ökade koncentrationer av L-dopa i blodet vilket leder till att en större mängd finns tillgänglig för hjärnan. Efter några års behandling med L-dopa utvecklar många av patienterna biverkningar i form av ofrivilliga rörelser. Dessa tros bero på att tablettbehandlingen orsakar svängande L-dopa koncentration som leder till en oregelbunden stimulering av hjärnans nervceller.

Inom Neurokirurgin utvecklades under 1980-talet en metod för implantering av elektroder i hjärnan för elektrisk stimulering och med positiva effekter på patienternas motoriska symptom. Metoden kallas för deep brain stimulation, DBS (djunghjärnstimulering). DBS operationer utförs med stereotaktisk teknik, vilket är en mycket noggrann operationsmetod för placering av elektroder i olika strukturer i hjärnan. Vid Parkinson’s sjukdom är placeringen vanligast i en nervcellskärna som heter nucleus subthalamicus, STN. Kunskapen om verkningsmekanismerna vid DBS är idag o tillräcklig.

Syftet med forskningen i denna avhandling var; att undersöka om enzymhämmare ger betydande koncentrationsvariationer av L-dopa i hjärnan; att kartlägga hur mycket av L-dopa medicineringen passerar över blod-hjärnbarriären; att undersöka eventuella samspel mellan L-dopa och DBS; att undersöka om DBS påverkar frisättningen av signalsubstanser i hjärnan; att undersöka hur stor del av vävnaden runt en mikrodialyskateter omfattas av dialysprovtagningen.

I samtliga arbeten har mikrodialystekniken använts, detta är en metod som gör det möjligt att kontinuerligt mäta olika substanser som tex läkemedel i både vätskor och vävnader. En mikrodialyskateter består av en mycket tunn plasxtärl med ett genomsläppligt dialysmembran i spetsen med ett flöde av substanser över membranet. Substanserna samlas upp som ett dialysat i en liten behållare och analyseras.

Vi har visat att enzymhämmaren entacapone ökar tillgängligheten av L-dopa i nervsystemet men att den också ger ökade koncentrationsvariationer och att preparatet skall användas i en kortare tid. Vi har visat att ca 18% av i blodet tillförd L-dopa återfinns i nervsystemet och att det finns ett samspel mellan L-dopa medicinering och DBS vilket delvis kan förklara varför man kan minska L-dopa medicineringen efter en DBS operation. Forskningen har även visat att DBS har effekt på olika signalsubstanser i hjärnan, ffa L-dopa, dopamin och GABA. Vi har visat att med stereotaktiska operationer kan man på ett säker sätt med mikrodialysteknik mäta kemiska ämnen i mycket små strukturer i hjärnan, hur stort område runt mikrodialyskatetern dialyseras och att mikrodialyskatetrarna är placerade i det område som vi velat studera.
ACKNOWLEDGMENTS

I wish to express my sincere gratitude to everyone I have worked with during this research, my colleagues, staff and patients. This thesis would not have been possible without the involvement of a large number of people and I would like to thank the following:

Nil Dizdar, my Supervisor, for your teaching, support, constant enthusiasm and encouragement throughout these years. We have had a lot of discussions over the years and you have always found the time to work with me. It has been a pleasure working with you and to see your never ending enthusiasm regarding Parkinson’s disease.

Jan Hillman, my Co-supervisor and Head of the Department of Neurosurgery, for your never ending enthusiasm for research and Neurosurgery in general. For providing me with the support, time and a stimulating atmosphere in which to conduct my research. Thank you for your help in reviewing the manuscript.

Lars Erik Augustinsson, colleague and pioneer in DBS surgery, for teaching me the fundamentals in stereotactic neurosurgery. We have had a lot of interesting discussions over the years and you have always been supportive of me.

Anita Kullman, co-author and collaborator, thank you for your knowledge and always being there for me.

Karin Wårdell, co-author, for your help and enthusiasm in Neuroscience research, especially DBS and for your help in reviewing the manuscript and for your never ending encouragement.

Elin Diczfalussy, co-author, for your help in understanding engineering issues, computer programs and explaining things in a way that I can easily understand.

Maria Nord, for your friendship and collaboration in the articles.

Johan Richter, friend and colleague in functional neurosurgery, for helping me with microdialysis during surgery.
Staff and colleagues at the Department of Neurosurgery. A special thanks to Patrik Sturnegk, for always finding some space in the schedule for my research.

Sussanne A. Larsson for excellent help with manuscript layout.

Nora Östrup, linguistic revisor of the thesis.

My family, Camilla, Emmy, Eric for your support and for always being there for me.
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