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FUNCTIONAL MRI CHARACTERIZATION OF ANIMAL MODELS OF PARKINSONISM

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Functional MRI characterization of animal models of Parkinsonism
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To the lab animals who have participed in the making of this work. I hope the findings obtained outworth your sacrifice.

ABSTRACT

Parkinson's disease (PD) is the second most common neurological disorder. It is characterized by the progressive development of motor symptoms - bradykinesia, resting tremor, muscular rigidity and difficulty with postural control - which serve as criterias for its clinical diagnosis. However, there is a need for biomarkers to detect PD early before the appearance of the symptoms, but also to evaluate efficacy of treatments. Such biomarkers would also to evaluate the translational value of models of the disease. In recent years, magnetic resonance imaging (MRI) has been used by researchers to identify biomarkers of PD in the patients' brain. One MRI method that is gradually becoming more popular is resting-state functional MRI (rs-fMRI). It consists in tracking the activity of brain by acquiring the MRI signal of the brain over time for several minutes while the patient is at rest, i.e. when he/she tries not to think about anything in particular. Compared to task-based fMRI, it is advantageous for studying PD as patients have problems to perform tasks, both because of motor symptoms but also cognitive symptoms which are common in PD.

In this thesis, after successfully demonstrating the translational value of rs-fMRI by comparing a set of functional networks in naive Sprague-Dawley and healthy human subjects (paper I), several rat models of parkinsonism were characterized. These models consisted in a well-established model, the unilateral 6-hydroxydopamine (6-OHDA) model (paper II), and two progressive models of parkinsonism, the alpha-synuclein adeno-associated virus overexpression model, a genetic model (paper III), and the β-sitosterol-β-D-glucoside model, a new toxin-based model (paper IV).

By acquiring rs-fMRI datasets and analysing them using seed-based correlation analysis, functional connectivity maps were generated. We could reproducibly demonstrate that sensorimotor corticostriatal functional connectivity is increased in the 6-OHDA lesioned animals compared to their control counterparts, while in models with milder parkinsonian pathology, the sensorimotor corticostriatal functional connectivity is decreased. We therefore emit the hypothesis that there is a U-shaped function describing corticostriatal functional connectivity relative to the level of striatal dopaminergic innervation. We also observed in both models of mild parkinsonism a reinforcement of negative functional connectivity between the prefrontal cortex, in particular the orbital cortex, and the primary somatosensory cortex compared to their healthy counterparts.

These results demonstrate that rs-fMRI is a valid method to observe alterations in the brain related to parkinsonism in animals and that both motor and non-motor areas of the brain are affected by the loss of dopaminergic neurons. Further investigations must be conducted to understand the mechanisms involved in these changes and evaluate their translational value.

LIST OF SCIENTIFIC PAPERS

I. **Default Mode Network, Motor Network, Dorsal and Ventral Basal Ganglia Networks in the Rat Brain: Comparison to Human Networks Using Resting State-fMRI.** By: A. Sierakowiak, C. Monnot, S. Nikkhou-Aski, M. Uppman, T.Q. Li, P. Damberg and S. Brené (2015). PLoS One 10(3):e0120345.

II. **Asymmetric dopaminergic degeneration and levodopa alter functional corticostriatal connectivity bilaterally in experimental parkinsonism.** By: C. Monnot, X. Zhang, S. Nikkhou-Aski, P. Damberg and P. Svenningsson (2017). Experimental Neurology 292: 11-20.

III. **Overexpression of human alpha-synuclein in the Substantia Nigra of rats alters functional connectivity in motor and default mode networks.** By: C. Monnot, J. Zareba-Paslawska, J. Perens, P. Damberg and P. Svenningsson. (manuscript)

IV. **Resting-state functional MRI characterization of the beta sitosterol beta-D-glucoside rat model of parkinsonism.** By: C. Monnot, M. Kalomoiri, P. Damberg, J.M. Van Kampen, H. Robertson and P. Svenningsson. (manuscript)

CONTENTS

LIST OF ABBREVIATIONS

6-OHDA	6-hydroxydopamine
AAV	Adeno-Associated Virus
α-syn	Alpha-synuclein
ALS-PDC	Amyotrophic lateral sclerosis-parkinsonism-dementia complex
BOLD	Blood Oxygen Level Dependence
BSSG	Beta-Sitosterol-Beta-D-Glucoside
DAT	Dopamine Transporter
DMN	Default-Mode Network
DRT	Dopamine Replacement Therapy
EPI	Echo-Planar Imaging
FA	Fractional Anisotropy
FC	Functional Connectivity
fMRI	Functional MRI
FSL	FMRIB Software Library
GABA	Gamma-aminobutyric acid
GPe	External part of Globus Pallidus
GPi	Internal part of Globus Pallidus
L-DOPA	Levodopa
LID	L-DOPA induced dyskinesia
MD	Mean Diffusivity
MFB	Medial Forebrain Bundle
MRI	Magnetic Resonance Imaging
MSNs	Medium Spiny projection Neurons
PD	Parkinson's disease
QSDR	Q-Space Diffeomorphic Reconstruction
rs-fMRI	Resting-state fMRI
SN	Substantia Nigra
SNc	Substantia Nigra pars compacta
SNr	Substantia Nigra pars reticulata
SPM	Statistical Parametric Mapping Toolbox
STN	Subthalamic nucleus
Str	Striatum
VTA	Ventral Tegmental Area

1 INTRODUCTION

1.1 PARKINSON'S DISEASE – PATHOGENESIS AND SYMPTOMATOLOGY

Parkinson's disease (PD) is the second most common neurodegenerative disorder (Kalia and Lang, 2015). It is also the most common movement disorder. PD is characterized and diagnosed by the progressive development of motor symptoms: bradykinesia, resting tremor, muscular rigidity and difficulty with postural control. There is a progressive loss of dopaminergic neurons in the Substantia Nigra (SN) of PD patients and this loss is responsible for most of the motor symptoms found in PD. Another well-known pathological characteristic is the presence of Lewy bodies, inclusions of misfolded proteins, mainly alpha-synuclein (α-syn).

Around five-ten percent of the PD cases are familiar and have an identified genetic origin. Many different mutations cause PD. These includes mutations in α-syn, Parkin and LRRK2. In addition, there is an association between mutations in certain genes, such as GBA, and the likelihood to develop PD. However, in most instances, there is no identified genetic cause for the development of PD and these cases are called sporadic or idiopathic.

Braak et al. (2000 and 2003) have hypothesized, based on the pathological examinations of post-mortem samples from PD patients and the occurrence of α-syn inclusions (corresponding to Lewy neurites/bodies) , that the disease consists of several stages:

- A preclinical/premotor stage in which α-syn inclusions are mostly present in the olfactory nerves, the enteric and autonomic nervous systems and brain stem nuclei. Common symptoms of this stage are hyposmia, REM sleep disorder, obstipation and depression.
- A clinical stage where motor symptoms are evident and the patient get her/his diagnosis. At this stage, Lewy bodies are also present in the midbrain including the dopamine neurons of SN.
- An advanced stage where Lewy bodies are present in most of the brain including cerebral cortex. Dementia and hallucinations are more common at this stage.

The motor symptoms of PD are treated with agents that stimulate dopamine neurotransmission (Kalia and Lang, 2015). In particular, the dopamine precursor levodopa or L-DOPA is highly efficient. To avoid that L-DOPA is metabolized in the periphery, causing nausea and hypotension, it is combined with a peripheral dopamine decarboxylase inhibitor. This combination enables most of the L-DOPA to produce dopamine in the nigrostrial system of the brain. Diverse strategies, mainly MAO B or COMT inhibitors, are used to strengthen and prolong its effect. Unfortunately, in a large proportion of PD patients, L-DOPA causes side effects. One of the main side effects which often appears after some years of L-DOPA treatment is involuntary movements, often referred to as L-DOPA induced dyskinesia (LID).

1.2 CIRCUITRY OF THE BASAL GANGLIA

1.2.1 The basal ganglia

The basal ganglia are subcortical brain regions which form loops with the thalamus and the cortex (Gerfen and Surmeier, 2011). These loops are usually called cortico-basal ganglia-thalamic loops. The structures of the basal ganglia include the striatum (Str), the globus pallidus, the subthalamic nucleus (STN) and the SN (Fig 1).

These regions are structurally and functionally interconnected. Str receives major excitatory glutamatergic inputs from the cortex and the thalamus along with dopaminergic inputs from the pars compacta of the substantia nigra (SNc). Str projects directly to the pars reticulata of substantia nigra (SNr) and the internal part of globus pallidus (GPi). Str also projects, indirectly to these regions, via SNr, via the external part of globus pallidus (GPe) and the STN (Fig. 1). There are several feedback loops within the basal ganglia. Moreover, STN receives direct input from the cortex. SNr and GPi send projections to the thalamus, which, in turn, project to cortex to regulate movements and cognitive processes.

1.2.2 The striatum

Str is composed in humans of three subregions, the nucleus accumbens or ventral Str, the caudate nucleus and the putamen. In rodents, the caudate and the putamen cannot be distinguished and is therefore often called the caudate putamen in these species. An alternative name is the dorsal Str.

Str is thought to have different roles depending on the subregions. The ventral Str and in particular the nucleus accumbens is a center for the control of reward, motivation and goal oriented behaviours, while the putamen is mainly involved in the modulation of movements and the caudate nucleus in the modulation of cognitive processes and eye movements (Gerfen and Surmeier, 2011).

As aforementioned, Str receives afferent glutamatergic axons from the cortex, but also from the thalamus. It receives dopaminergic axons from the SNc and the Ventral Tegmental Area (VTA), respectively in the dorsal Str and the ventral Str (Dahlström and Fuxe, 1964; Gerfen and Surmeier, 2011). Recent findings have also indicated that in rodents the dorsal Str receives afferent GABAergic axons from a distinct population of neurons in the GPe (Mallet et al., 2012).

Str is composed to 90-95 percent of inhibitory GABAergic Medium Spiny projection Neurons (MSNs). Other neuronal populations are large cholinergic interneurons and GABA interneurons. There are several classes of the later, including Parvalbumin-positive fast spiking interneurons, nitric-oxide synthase-positive interneurons and calretinin-positive interneurons.

1.2.3 Striatum, Medium Spiny Neurons and the direct and indirect pathways

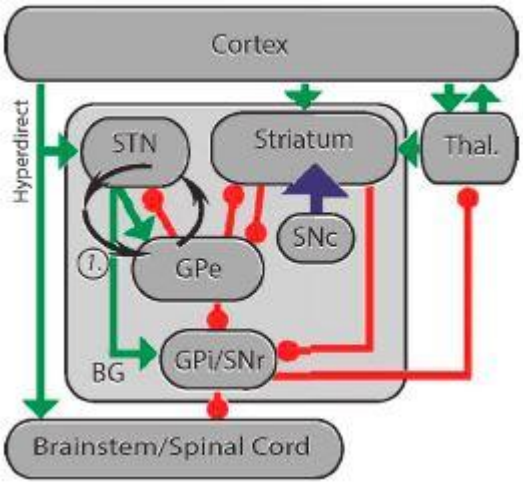

Fig.1: Model of the cortico-basal ganglia-thalamic loops. The red arrows indicate GABAergic connections, green arrows glutamatergic and blue arrows dopaminergic connections. Figure adapted from Ellens et al., 2013.

MSNs can be divided in two major types. MSN expressing D1R type receptors, which are mainly stimulatory, and MSNs expressing D2R type receptors, which are inhibitory (Gerfen and Surmeier, 2011). These two populations of neurons project their axons to two different regions, with the D1 MSNs being part of the direct striatonigral pathway. D1-mediated stimulation of this pathway dishinibits the thalamic neurons projecting to the cortex.. D2-mediated inhibition of the D2 MSNs, which are part of the indirect striatopallidal pathway, disinhibit the GABAergic neurons of the GPe and reinforce the inhibition of GABAergic neurons to the GPi and SNr. This disinhibition activates thalamic neurons projecting to the cortex.

Thus, tonic dopamine levels in the Str modulate the excitability of both D1 MSNs and D2 MSNs in opposite directions, the more dopamine, the more D1 MSNs are excitable and the less D2 MSNs are excitable. However, dopamine causes motor stimulation via both the direct and indirect pathways.

1.2.4 Symptoms of PD and loss of dopamine

As mentioned earlier, the loss of dopaminergic neurons in SNc projecting to putamen is the major cause of the motor symptoms of PD In addition, several network effects have been found as a result of dopamine depletion. In healthy conditions, the activity of the basal ganglia is not synchronized with the motor cortex activity (Bar-Gad et al, 2003), but a synchrony develops upon chronic depletion of striatal dopamine (Dejean et al, 2012).

Moreover, beta oscillations, which are transiently occurring in the cortex and basal ganglia when subjects are purposefully maintaining a certain movement (Jenkinson and Brown, 2011; Leventhal et al, 2012), are enhanced and are more coherent in PD patients and animal models of PD (Mallet et al, 2008; Brazhnik et al, 2012). Indeed, the presence of beta oscillations is associated with bradykinesia and rigidity in PD (Little et al., 2012; Kühn et al., 2009) as well as upon dopamine depletion in animals (Mallet et al, 2008).

Several different PD therapies restores dopamine neurotransmission and improves the movement disorder of PD patients. Treatments with dopaminergic agents or Deep Brain Stimulation seem to decrease the synchrony observed in the parkinsonian basal ganglia (Heimer et al., 2002; Burkhardt et al., 2007). The same treatments also proved to be effective

at reducing the degree of beta oscillations in the basal ganglia of PD patients (Brown et al., 2001; Cassidy et al., 2002; Levy et al., 2002; Priori et al., 2004, Kühn et al., 2008; Kühn et al., 2009 Bronte-Stewart et al., 2009).

In addition to accumulated long-term therapy with moderate to high doses of L- DOPA, the development of LIDs requires a substantial loss of striatal dopaminergic terminals (Schneider, 1989; Di Monte et al, 2000; Cenci and Lindgren, 2007). It has been reproduced both in rodents and non-human monkeys after repeated L-DOPA and has been linked to an abnormal type a plasticity (Cenci and Lindgren, 2007). In PD patient, LID appears rapidly in younger patients whom have a more plastic nervous system (Schrag et al., 1998). LIDs involve many neurotransmitters other than dopamine and are often difficult to treat. Current therapies target glutamate or serotonin receptors.

1.3 ANIMAL MODELS OF PARKINSONISM

In this subsection I will focus on the toxin and genetic animal models of Parkinsonism which are studied within the frame of this thesis.

1.3.1 The 6-OHDA rat model

The 6-hydroxydopamine (6-OHDA) model to deplete nigrostriatal dopamine transmission was developed by Urban Ungerstedt at the Karolinska Institutet (Herrera-Marschitz et al., 2010). 6-OHDA is a toxin targeting the monoaminergic neurons, such as the dopaminergic neurons, but also noradrenergic and serotonergic neurons. 6-OHDA enters the monoaminergic neurons via the monominergic transporters such as the dopamine transporter (DAT). 6-OHDA is a molecule that resembles dopamine and that is changed into oxidative free radicals once in the soma provoking the neurons' death.

6-OHDA does not cross the blood-brain-barrier and must therefore be injected directly into the brain. There are thus two common types of 6-OHDA rat models of PD, the model where 6-OHDA is injected in the Str and the model where 6-OHDA is injected in the medial forebrain bundle (MFB), a white matter structure which carries among other axons the dopaminergic axons from the SNc to the Str. The intrastriatal model provokes a neurodegeneration over two-three weeks in the SNc resulting in a partial loss of dopaminergic neurons, while intra-MFB model provokes a near complete loss (about 95%) of the dopaminergic neurons in the SNc within a few days. This intra-MFB model requires protection of noradrenergic axons in the MFB originating from the locus ceuruleus using a substance like the noradrenaline reuptake inhibitor, desipramine. The injection of 6-OHDA is done unilaterally as a bilateral injections provoke a high mortality.

6-OHDA-lesioned animals are very useful to assess anti-parkinsonian activity of various compounds. They react to L-DOPA and other antiparkinsonian treatments, such as apomorphine, by contralateral rotations which are easily quantified. They also respond in similar ways to high-frequency stimulation of the STN as PD patients with Deep Brain Stimulation. Furthermore, the partial intrastriatal 6-OHDA model is not only used to study

anti-parkinsonian activity of various compounds, but also to assess neuroprotective strategies. In addition to study stimulatory anti-parkinsonian properties of compounds, the intra-MFB model along with repeated dosage of L-DOPA causes involuntary movements similar to LID in PD patients (Cenci and Lindgren, 2007). LIDs are evaluated in rodents using the Abnormal Involuntary Movements Score, which consist in observing the animals during five minutes while they are under the effect of L-DOPA and measure how often the animals demonstrate involuntary movements from the limbs, the trunk and the face.

From a mechanistic standpoint, reinforced beta-oscillations similar to those in PD patients have been observed in the 6-OHDA model (Mallet et al., 2008). It has also been shown that glutamate release by the corticostriatal neurons is increased in 6-OHDA rats compared to controls (Alvarsson et al., 2015). Majors drawbacks of the 6-OHDA model are that it does not present with any Lewy-body inclusions and that the model is not progressive.

1.3.2 Human α-syn overexpressing adeno-associated virus (AAV) rat model

To study the influence of the α-syn on the neurodegeneration of dopaminergic neurons, human α-syn is introduced in neurons of SNc using a viral vector (Kirik and Björklund, 2003). For our studies, AAV-α-syn is overexpressed by dopaminergic neurons following the injection in the SNc (Caudal et al., 2015). This model provokes the death of these neurons in a progressive manner and the neurodegeneration cease after 12 weeks. The extent of the loss of dopaminergic neurons is around 50-60% and these animals express a mild parkinsonian phenotype. Stronger phenotype and neurodegeneration have been reported using a mutated human α-syn (Van der Perren et al, 2015). Lewy-body inclusions have been observed in the model.

1.3.3 The β-sitosterol β-D-glucoside (BSSG) rat model

BSSG is a substance found in cycad seeds, a plant that is suspected to be the cause of amyotrophic lateral sclerosis-parkinsonism-dementia complex (ALS-PDC), also known as Guam disease. ALS-PDC presents itself in two forms, one that resembles amyotrophic lateral sclerosis and another that resembles PD. When BSSG is fed for sixteen weeks to Sprague-Dawley rats, they progressively develop a parkinsonian pathology which develop over several months (Van Kampen and Robertson, 2017). The animals first present an olfaction deficit similar to anosmia (from the twelfth week of feeding and onward), an early sign of PD, before to develop motor deficits (from about the twentyfourth week). At a later stage, around the tenth month, animals have memory impairment, similar to dementia which develop in some PD patients at a late stage. α-syn aggregates can be found in areas related to the phenotype presented by animals with aggregates in the olfactory bulb at first, before to be found in Str and SN and finally hippocampus and prefrontal cortex (at 10 months). Interestingly, the locomotor deficits and nigral dopaminergic neurodegeneration appear in an asymmetric fashion before to have both hemispheres equally affected (Van Kampen et al., 2015). Though very little has been done in this model so far, all the aspects mentioned above make the BSSG rat model a promising tool to study PD pathology.

1.4 MAGNETIC RESONANCE IMAGING

1.4.1 General principle

Magnetic resonance imaging (MRI) is a set of techniques developed in the 1970s and 1980s to obtain pictures of the content of an object based on the phenomenon of nuclear magnetic resonance. It allows primarily to map the local magnetic properties of soft tissues, which are rich in hydrogen. MRI is particularly appropriate to study the brain and its pathologies as high amounts of water and fat, both rich in hydrogen nuclei (protons), are present, thus giving a strong MRI signal. Also, MRI does not have any known negative effect on people's health. Finally, Numerous information can be obtained using MRI from the brain of patients such as the volume of different brain areas, the presence of tumors or hemorrhages, the presence of iron accumulation or, more importantly within the scope of the current thesis, knowledge about the free water diffusion properties (using diffusion MRI) and neuronal activity (using functional MRI).

1.4.2 Functional MRI

Functional MRI (fMRI) consists in observing the neuronal activity by using a phenomenon called BOLD. BOLD stands for Blood Oxygen Level Dependence which is the signal obtained due to the variation in concentration of oxyhemoglobin following an intense neuronal activity. By recording the BOLD signal over time, one can determine the location of intense neuronal activity during the performance of a task for example.

1.4.3 Resting-state fMRI

1.4.3.1 Principle

Resting-state fMRI (rs-fMRI) is the use of fMRI when no particular task is performed by the subject. In these experiments, patients are asked to lie down, close their eyes and not think about anything specific. This is a particularly interesting approach for PD as it is often difficult for patients to execute some tasks, in particular in the later stages of the disease, or if they suffer from dementia.

A common method to analyze rs-fMRI is called seed-based correlation analysis and consists in calculating the correlation between a region of interest, also called a seed, and the rest of the brain. This correlation is also called the functional connectivity (FC). Other methods exist such as independent component analysis, which decompose the signal from the whole dataset into networks of brain areas with coherent signal over time, or algorithms based on graph theory.

1.4.3.2 The default-mode network (DMN)

DMN is a collection of regions which were first characterised by the fact that they were active when patients were not performing tasks, i.e. between tasks during fMRI scans. It was first thought to be areas that are active when the person is inactive and was therefore given its name of default mode. It was later proven that DMN can be activated using tasks involving

introspection, autobiographical tasks or social working memory. DMN regions have been characterised using independent component analysis but can also be detected using correlation analysis with a seed placed in the posterior cingulate cortex.

The DMN is composed of the infralimbic cortex, the orbital cortex, the cingulate cortex, the precuneus, the dorsal hippocampus and the retrosplenial cortex. It is present in both humans, non-human primates but also rodents (Lu et al.,2012; Sierakowiak et al.,2015). Since its discovery, the study of DMN and its involvement in different neurological disorder has grown exponentially. DMN was the first resting-state network to be described but several of them have since been described, involving about every area of the forebrain (visual, auditory, evecutive function, salience, sensorimotor, etc).

1.4.3.3 Resting-state fMRI in PD patients

In PD patients, many experiments have been conducted using seed-based correlation analysis, with seeds placed in the Str, the motor cortex and other regions. The results are contradictory. Some studies conducted with seeds placed in the putamen found a decrease of FC between the putamen and the cortex in PD patients compared to the control group (Palmer et al., 2010, Luo et al., 2014). However, other studies found the opposite (Yu et al., 2013; Kwak et al., 2010; Agosta et al., 2014). To add complexity, a study even found that when they place a seed in the posterior putamen, they saw a decrease in connectivity towards the motor cortex, but when the seed was placed in the anterior putamen they observed an increase of connectivity (Helmich et al., 2010). Studies with seeds placed in the STN showed an increase of FC between the STN and the sensorimotor cortex (Baudrexel et al., 2011; Kurani et al., 2014; Fernandez-Seara et al., 2015; Kahan et al., 2014). These discrepancies were found despite that all these results were found while patients were OFF- dopamine replacement therapy (DRT).

Tahmasian et al. (2015) argued in a review about the importance to study PD patients OFF-DRT. They reviewed results obtained in studies conducted ON-DRT and studies OFF-DRT or on de novo patients and observed that the findings observed were quite different. They also reviewed the few studies conducted on patients, where the patients were scanned both OFF and ON-DRT and pointed out the fact that DRT had an effect on functional networks. They therefore concluded that fMRI studies investigating the functional networks of patients relative to symptoms of PD should be studied OFF-DRT as DRT seems to alter the results of the studies.

A recent study by Badea et al. (2017) compared results from three independent cohorts of PD patients and controls and were unable to reproduce FC results between the three cohorts. By making further tests they concluded that the cause for their inability to reproduce their findings were due to the heterogeneity of PD between patients, rather than technical differences.

Despite the fact that more than hundred fMRI studies have been published in the past ten years, the first meta-analysis focused on rs-fMRI with seed-based correlation analysis

conducted in PD patients was only published this year (Ji et al., 2018). This publication focused on corticobasal ganglia thalamocortical network. It was found that the most significant finding was an increased FC from the different seeds of the network (putamen, caudate, thalamus, primary motor cortex, supplementary motor area, STN, etc) to the left pre- and postcentral gyrus (corresponding to primary motor and primary somatosensory cortices respectively) in PD patients.

1.4.3.4 Resting-state fMRI in the 6-OHDA rat model

There are only three published articles focusing on the use of rs-fMRI in animal models of parkinsonism including paper II. All three articles are focused on characterizing the 6-OHDA model. By using graph theory methods, Westphal and colleagues (2017) found a decrease in "functional connectivity" primarily in the hemisphere ipsilateral to the lesion in 6-OHDA lesioned rats and in particular in motor, somatosensory and orbital cortices, while paper II (Monnot et al., 2017) and Perlbarg and colleagues (2018) found an increase in corticostriatal FC in these animals using seed-based correlation analysis. The difference in conclusions between the first and the two other article is mainly due to the fact that the two methods employed define "functional connectivity" in a different way, one consider all connections and attribute it to the region while the other quantify it for each connection separately, so the two measures are not equivalent.

1.4.4 Diffusion MRI

1.4.4.1 Principle

Diffusion MRI is becoming more popular to study diverse neurological disorders and in particular PD. Diffusion MRI measures the diffusion of water molecules in an organ, most commonly the brain. Diverse methods exist to calculate, based on the images obtained, an estimate of the local diffusion properties of the tissues. The most common approach is Diffusion Tensor Imaging, which, based on at least 6 diffusion directions, calculates several metrics. The two best known metrics are Fractional Anisotropy (FA) and Mean Diffusivity (MD). MD represents how much the molecules can diffuse in the tissue, while FA is a scale for whether the molecules diffuse equally in any direction (FA=0) or are restricted to a preferred direction (FA=1).

Those properties are particularly interesting for neurological studies as the white matter typically has a high FA and a low MD, because of its structure, while ventricles have a low FA and a high MD as water diffuse freely in cerebrospinal fluid. Diffusion MRI can therefore indicate the state of tissues and detect lesions and aggregates as a response to neurodegeneration.

1.4.4.2 Diffusion MRI in PD patients

Many studies have been conducted in the past few years using diffusion MRI and two meta-studies were also conducted (Cochrane and Ebmeier, 2013; Schwarz et al., 2013). These meta-studies found only one consistent finding, an FA decrease in the SN of PD patients. One

of the meta-study also found an increase of MD in the same region (Schwarz et al., 2013). Both would be consistent with the dopaminergic neurodegeneration observed in patients, as the region loses the neurons and therefore their axons and their large dendritic trees. Other observations made by diverse studies, are a loss of FA and/or an increase in MD along a number of white matter tracts (Scherfler et al., 2013; Kamagata et al., 2013; Kamagata et al., 2014; Kim et al., 2013). However there does not seem to be any consistency on the white matter tracts where the changes are observed. These changes could be secondary to the disease and vary depending on the particularities of the cohorts used.

1.4.4.3 Diffusion MRI in models of parkinsonism

Diffusion MRI was performed in-vivo on a striatal injection 6-OHDA rat model (Van Camp et al, 2009). An increase in FA as well as a decrease in diffusion values was detected in the SN of the animals ipsilateral to the lesion. However Boska et al (2007) have observed a decrease in FA and a increase in diffusitivity in the SN several days after a MPTP intoxication in mice, another toxin model of parkinsonism, compared to mice treated with a sham solution. More recently, a study demonstrated by using diffusion kurtosis imaging, an alternative diffusion MRI paradigm to diffusion tensor imaging, on a genetic mouse model overexpressing human α-syn that mean kurtosis increases in SN, caudate, and a number of other area already at three months old compared to the control animals (Khairnar et al., 2017).

2 AIMS

1. To deepen knowledge of the function of basal ganglia and its relation to cortex.

2. To characterize different models of parkinsonism with rs-fMRI.

    i. To characterize functional alterations in individual models of parkinsonism.

    ii. To find common patterns of changes among the different models.

3. To determine functional changes related to the loss of dopamine in rodents.

3 METHODOLOGICAL ASPECTS

3.1 BEHAVIOURAL TESTING

To ensure that a model is successfully created before to acquire and analyse fMRI datasets, one may use behavioral tests to detect phenotypical changes in the animals. To detect the parkinsonian phenotype in rats, a number of tests exist, in particular to quantify motor deficits.

In paper IV, three different tests have been used, two which focus on motor aspects of parkinsonism and one focusing on traits related to anxiety and depression. These tests are, in the order of use in the papers, the open-field test, the ledged-beam walking test and the sucrose preference test.

3.1.1 Open-field test

The open-field test is a generic test to characterize the phenotype of an animal, several metrics can be obtained from this test. Regarding motor abilities relevant to parkinsonism, one can measure the distance traveled during the duration of the test as well as the mean velocity when animals move. One can also measure the time the animals spend exploring the edges as a measure of thygmotaxis, a behavior related to anxiety and depression. One may also measure the frequency at which the animals do certain behaviors such as rearing, freezing, etc.

3.1.2 Ledged-beam walking test

The ledged-beam walking test is a more specific test where the experimenter measures the number of errors the animals make with each hindpaw while walking along the beam. From the number of errors made over three trials, one can determine whether motor phenotype related to striatal activity is involved and if there is a side with a larger loss in striatal dopamine for example.

3.1.3 Sucrose preference test

The sucrose preference test is made to detect anhedonia. It consists in having animals placed individually in a home cage in presence of two bottles, one filled with water and the other with a sucrose solution. Healthy animals prefer the sucrose solution. In case of anhedonia, animals will be more indifferent to the sweet solution. Anhedonia is an indicator of depression, which is common among PD patients.

3.2 FMRI ACQUISITION IN RATS

3.2.1 Animal preparation

In order to obtain MRI data of good quality, it is important to setup the animal in a good way before to transfer it into the scanner. Many aspects are to be considered. Animals need to be in a stable state during the whole duration of the MRI scan. To ensure this, animals have their breathing rate, temperature, heart pulse and blood oxygen saturation monitored. Also, animals are anesthetised in order to keep them calm during the scan. Therefore, their temperature needs to be maintained at 37 degrees.

To obtain the best signal to noise ratio for BOLD signal, it is of particular importance to avoid vasodilation in order to detect changes in blood flow and oxygenation. It is therefore important to avoid halogenated gas anesthesia such as isofluran. Unfortunately isofluran is the main anesthetic agent used during MRI scans as it is appropriate to keep a good control over the depth of animals' anesthesia. Given this situation, medetomidine has been used in the past years to sedate animals during fMRI examination. Medetomidine is vasoconstricting and thus allows to obtain a strong BOLD signal, however, to start the sedation, animals are to be first anesthetised with isofluran or used combined with ketamine. Lu and colleagues (2012) have proposed a protocol for which animals are set to sleep with isoflurane before to give a bolus of medetomidine and then reduce isoflurane to 0.25% of the gas mixture. One must then wait for 90 minutes for the body to eliminate isofluran from the system before to do the fMRI examination. This is the procedure which was followed for papers II, III and IV, while in paper I, isoflurane was completely removed, but with a period of 90 minutes beteen the bolus of medetomidine and the star of the acquisition.

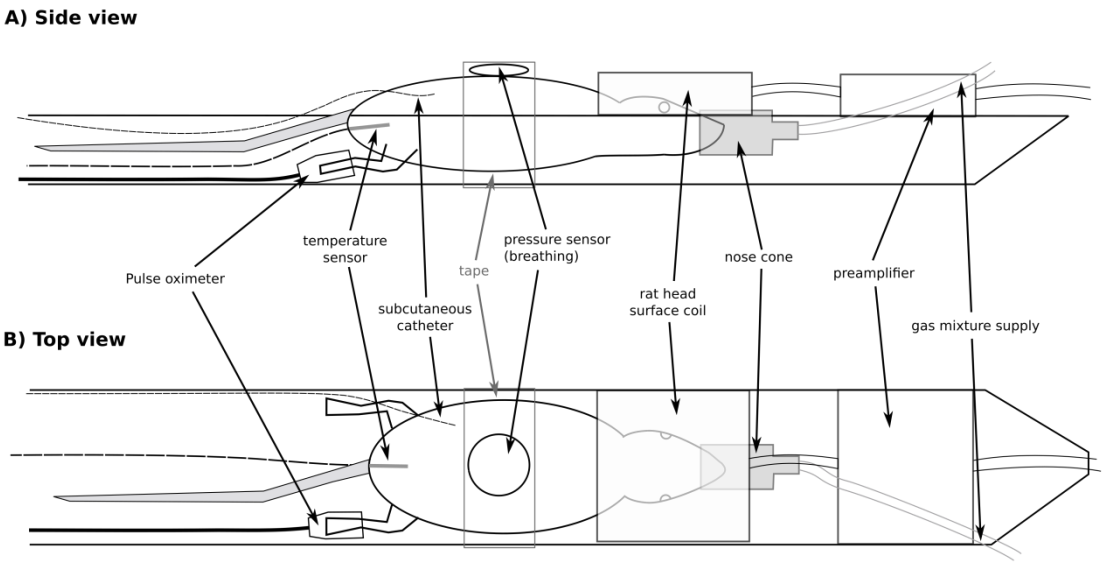

Fig. 2: Schematic representation of the setup for conducting resting-state functional MRI experiments in rats.

3.2.2 FMRI procedure

There are many ways to proceed with acquiring fMRI datasets. However, one must take care of eliminating confounders and mitigate artifacts which may affect the data quality and therefore the ability to interpret correctly the results obtained. One major effect to correct is the differences in signals depending on the location in the images because of a difference of sensitivity of the receiver surface coil, which is usually refered as the bias field of the coil. It is of particular importance for aligning the images of all subjects correctly. This is done by acquiring an extra structural image with each coil as a receiver, the surface coil and the volume coil.

Another artifact to take into account and mitigate is magnetic susceptibility artifact which consist in deformations of images as well as a loss of signal due to changes of magnetic susceptibility at the interface between different tissues, bones or air. This artifact also results in a loss of signal at this interface, in particular at high magnetic field. The prevalence of this artifact is dependent on the strength of the magnetic field of the MRI scanner and is particularly present when using gradient-echo echo-planar imaging (EPI), the classic MRI sequence for fMRI acquisition. It is therefore a major issue in preclinical MRI on small animals like rats or mice while it is quite limited in a clinical setup at 1.5T if the datasets are acquired appropriately.

Different strategies exist to reduce magnetic susceptibility artifacts. A first strategy is to reduce as much as possible the echo time of EPI, as the extent of the artifact depends on the echo-time. This is possible through two ways. To reduce the matrix size in the phase-encoding direction (which also limits the resolution of the images), but also to employ parallel imaging strategies to acquire the images. Parallel imaging consists in using the redundancy of the data acquired through the different channels of the surface coil to recreate an image from which only part of the information was obtained.

Another strategy that can be employed to mitigate susceptibility artifacts is to obtain the information about the magnetic susceptibility in the image and model the deformations. One can then invert the process to obtain the spatially corrected image. There are different ways to obtain this information. One can acquire an image similar to the results from EPI for different echo times and deduce fieldmap of the deformations by comparing the intensity in these images. This is the approach employed by the fieldmap tool in Statistical Parametric Mapping toolbox (SPM) (Wellcome Department of Imaging Neuroscience, University of London, London, UK) for MATLAB (Mathworks, Natick, Massachusetts, USA). Another way is to acquire the EPI datasets with the two opposite directions in phase-encoding gradients in an interleaved fashion over the image repetitions and then to estimate the deformations using the images for both directions. This is the approach employed by the topup tool (Andersson et al., 2003) in FMRIB Software Library (FSL)(Smith et al., 2004). It was first used to correct deformations in clinical diffusion MRI datasets which are acquired using spin-echo EPI. We decided to use this approach for papers III and IV and adapted the MRI sequence for EPI to allow us to use this approach.

Other changes have been made over time in the acquisition procedure between the different studies. In paper I, datasets were acquired in an axial direction (in the radiological convention, corresponding to coronal in biological convention) while in papers II, III and IV datasets were acquired in a coronal direction (horizontal direction in biological convention).

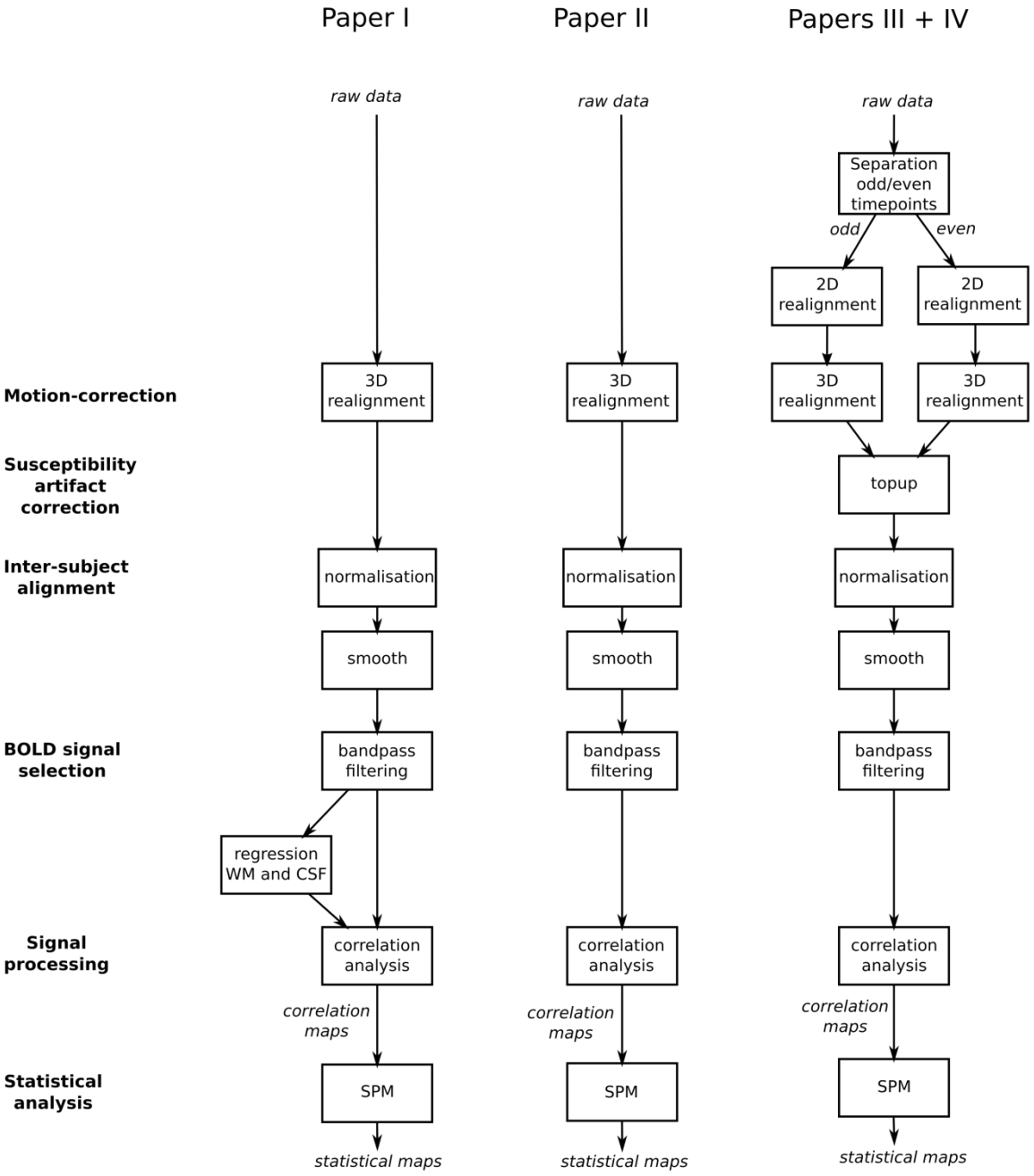

Fig. 3: Processing pipeline of resting-state functional MRI datasets for the different papers. SPM, statistical parametric mapping; CSF, cerebrospinal fluid; WM, white matter.

This change was made in order to cover the whole-brain in the shortest repetition time possible, hence maximising the amount of useful information acquired (by reducing the amount of extracerebral space in the images). Also the resolution between the first and other studies changed. The first study was made with voxel size equal to 0.5x0.5x1 mm^3 while others were made with voxel size equal to 0.65x0.65x0.65 mm^3. The matrix size was adapted accordingly. Total number of repetitions was also increased from three hundreds in paper I and II to eighteen hundreds in paper III and IV. This was done in order to decrease the variance of the results from seed-based correlation analysis within the same group by acquiring data for a longer period of time, thus increasing the chance to have animals in similar functional state in average and therefore increasing the sensitivity of the method when employed to models with an expected small effect size and milder phenotypical alterations.

3.3 RESTING-STATE FMRI ANALYSIS

3.3.1 Preparation of datasets for the analysis

Before to obtain the metrics to be compared between subjects, steps have to be taken to prepare datasets and reduce as much as possible the different sources of variance, which are the potential motion of subjects during the scan, the variation in position and size between different subjects, but also the exclusion of non-BOLD signals which may disturb the results such as the influence of heart pulse or breathing.

These steps are the same as those taken in clinical fMRI analysis though the influence of motion in rats is very small as the animals are anesthetised. Yet, motion in images can be detected over time due to a shift in gradient system properties due to heating over the course of the functional scan acquisition. This shift is small (less than 100 μm over the whole scan, most often around 30 μm) and progressive.

Also, during the pilot experiment conducted to prepare experiments for paper III, motion within slices could be detected in individual slices at occasional times but it did not affect the whole image, just a slice. We hypothetised that these motions occured due to breathing which affect locally the magnetic field based on the filling of the subjects' lungs with air. This was corrected by realigning separately each slice to a reference timepoint before doing the normal realignment procedure.

3.3.1.1 Motion correction - realignment of timeseries

Effects of motions are corrected using algorithms that compare images from one timepoint to a reference image by calculating the sum of square differences, which it tries to minimize by translating and rotating the image to realign it to the reference. It repeats these cycles of calculating the sum of square differences and transforming the image until it finds a minimum to this sum. This should correspond to the best alignment possible between the

images. This approach is implemented in both SPM's Realign, and in FSL's FLIRT (Jenkinson and Smith, 2001; Jenkinson et al., 2002).

3.3.1.2 Alignment between subjects - normalisation

The alignment of images between the different subjects to match them anatomically is similar to what is done for motion correction. The major difference is that besides rotating and translating images, it also allows shearing and zooming them. Also, as a final step, the algorithm involves some non-linear transformation, i.e. to locally deform images to improve the match with the template image. These algorithms are implemented in tools like SPM's Normalise and FSL's FLIRT (linear transformation) and FNIRT (non-linear transformation).

Following this step, functional datasets are spatially smoothed using a gaussian kernel to mitigate inconsistencies between the aligned datasets of different subjects. SPM's Smooth and the command fslmaths are tools used for this purpose.

3.3.1.3 Selection of BOLD signal - Band-pass filtering

BOLD is shown to have a frequency between 0.01 Hz and 0.1 Hz approximately. In order to remove signals not related to BOLD, one can apply a band-pass filter on the timecourses of each voxel for each subject. The band-pass filter removes the signal which have a different frequency by applying a Fourier transform to the timecourse and attribute the value zero to signals outside the desired frequency range before to use the inverse Fourier transform to obtain the filtered timecourses.

3.3.2 Seed-based correlation analysis

Seed-based correlation analysis consists in calculating the cross-correlation between the timecourse of a region of interest, also known as a seed, and the timecourse of every other voxel within an area of interest, in this case the whole brain. The timecourse of the seed correspond to the average of the timecourses of all voxels contained in the region of interest. In paper I and II, single voxels were used as seeds while in paper III and IV, a few voxels composed the seeds. This approach is implemented in a number of tools. Within the framework of this thesis, I used REST toolbox (Song et al., 2011) for MATLAB.

3.3.2.1 Statistics for seed-based correlation analysis

To obtain statistical results from seed-based correlation analysis, parametric statistical tests were performed on correlation maps obtained with the method described above. Statistical parametric mapping tools such as SPM or equivalent tools from FSL or other software distributions can be used. In SPM, one must use the factorial design tool which performs statistics based on a generalised linear model where the formula used to explain the variance is the following:

$$\mu = \beta * X + \varepsilon \qquad \textit{Eq. 1}$$

where μ is the dependent variable to be explained, X the matrix of the independent variables (i.e. group, gender, age, treatment, ...), β the parameters to be determined and ε the residual value which cannot be explained based on the independent variables provided. The algorithm tries to determine the values for β in order to minimise ε. μ for a normal distribution as in this case is the mean value.

Using this principle, one can perform analyses of variances and Student's t-tests to compare groups. Statistical tests are performed independently for each voxel of the images. One can then correct the probabilities of a false positive using a family-wise error correction or based on the size of cluster of statistically significant findings.

It is worth noting that correlation must be converted using a Fisher transform before to be used for parametric statistical tests in order to respect the requirements regarding the distribution of values which must follow a normal distribution.

3.4 POST-MORTEM MRI ACQUISITION OF RAT HEADS

MRI is a slow imaging method which is sensitive to motion. Therefore when one wants to obtain accurate images with particularly long scan times with small-animal MRI, one may opt for performing the experiment post-mortem. This allows to perform scans for very long periods of time, motion-free. This is particularly useful for acquiring high-resolution diffusion MRI datasets in rodents.

3.4.1 Rat head preparation for MRI scan

The main goal of preparing the rat head is to allow for scanning the brain of the animals with the smallest amount of artifacts in the images produced during the scan. Therefore, one must take into account all sources of magnetic susceptibility artifacts, but also reduce sources of noise and position the tissues so that the sample is immobile during the entire duration of the scans. The brains must also be preserved from any damage or physical deformation in order to allow a good alignment of structures between subjects and a good match to living animals equally. This last point is ensured by keeping the brains within the skulls, the natural shell to the brain.

Before to prepare the rat head for scanning, one must have the animal perfused with a paraformaldehyde solution intracardially in order to stabilise the tissues and allow for manipulation of tissues at room temperature without degradation.

To limit magnetic susceptibility artifacts, skin is removed, thus reducing surfaces where air bubbles are prone to form and heads are placed into perfluoropolyether (Fomblin, Solvay Solexis, Neder-Over-Hembeek, Belgium), a fluorinated oil, which has a magnetic susceptibility close to tissues' susceptibility, while being chemically inert and having no MRI signal. One must then try to extract as much as possible remaining air bubbles that may remain within the sample.

Further steps can be taken to ensure the absence of air by breaking the tympanic bulla, a part of the temporal bone of the skull which is located at the level of the midbrain and is usually filled with air.

To reduce the thermal noise in the images, one can reduce the number of electrical charges of the sample (ions) by removing all the extra tissues surrounding the skull. This has also for effect to reduce the size of the sample and therefore allows to reduce the field of view and the matrix size, resulting in a reduced scan time.

Finally, one may improve the scanning time drastically by adding some gadolinium-based contrast agent to the perfusion solution and in the post-perfusion bath. This has for effect to reduce dramatically the magnetic resonance relaxation times of the tissues, and therefore reduce the time necessary to scan the sample, allowing to achieve a higher spatial resolution and/or a larger number of diffusion directions in a diffusion MRI experiment for a same amount of time.

3.4.2 Diffusion MRI acquisition of rat heads

Acquiring diffusion MRI can be done with different MRI sequences. When applied in patients at the clinical level, one must reduce the duration of the scans and use fast sequences such as EPI to obtain the datasets. However, when acquiring datasets at very high magnetic field as in a preclinical setup, EPI results in images with limited image resolution or high image deformations due to susceptibility artifacts. To obtain images with a resolution equivalent in scale to the resolution of clinical images (roughly a factor ten in each dimension between humans and rats), one must consider slower methods such as a spin-echo multislice sequence (when acquiring without contrast agent) or a spin-echo 3D sequence (when acquiring with contrast agent). This change requires to acquire the images post-mortem as the typical duration of a spin-echo multislice scan for diffusion imaging with 6 directions at 150 µm isotropic resolution is around nine hours. Furthermore, in order to use superior methods to analyse diffusion MRI datasets such as connectometry analysis or Q-ball imaging, a large number of diffusion directions must be acquired. which prolonges the duration of scans.

Unfortunately, to obtain the same results as in-vivo, one must then increase the b-value of the diffusion paradigm as the perfusion with paraformaldehyde affects the diffusion properties of brain tissues (Dyrby et al., 2011). The b-value for images in paper II were obtained with a b-value of 1250 s/mm^2 while ideal value would be around 3000 or 4000 s/mm^2.

3.5 CONNECTOMETRY ANALYSIS IN DIFFUSION MRI DATASETS OF RODENTS

Different methods exist to analyse diffusion MRI datasets. The most common methods use diffusion tensor imaging to reconstruct the diffusion tensor from the datasets and then use the parameters extracted such as FA or MD to compare the different subjects, either directly between the subjects within the same areas or by using a probabilistic approach to reconstruct tracts and compare the properties of these tracts. Those two approaches have been

implemented in softwares such as FSL's TBSS (Smith et al., 2006) and FSL's FDT (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FDT) respectively.

A recently developed method, connectometry analysis, quantifies instead, for every subject, a given metric, such as FA, along white-matter tracts defined using a deterministic tracking algorithm on a template dataset. It then uses non-parametric statistical tests to define whether a significant relation is found (Yeh et al., 2016). In the case of paper II, a group comparison was made for the isotropic value of the diffusion along white matter tracts and found an increase along tracts corresponding to the nigrostriatal pathway in the hemisphere lesioned using 6-OHDA.

In order to conduct such an analysis, one must reconstruct the orientation diffusion functions from the diffusion MRI datasets within a common template, therefore requiring to normalise the datasets simultaneously with this reconstruction step. This is what is done using q-space diffeomorphic reconstruction (QSDR) which is provided in the software DSI Studio (Yeh and Tseng, 2011). Unfortunately, there is no standard template of rat brains for this method, so one must create his/her own template using diffusion MRI datasets. By using generalized q-sampling imaging (Yeh et al.,2010), quantitative anisotropy maps can be obtained, which can then be normalised to a common space and the map of the average quantitative anisotropy can be calculated. The quality of the template is then dependent on the quality of the alignment of fine white matter structures and the number of subjects that are used to calculate the average map.

Once the template for QSDR is created, one can run QSDR on the datasets to be analysed. Also one must have datasets going through QSDR from healthy animals in order to create a template from which tracts for connectometry analysis will be resolved.

4 RESULTS AND DISCUSSION

4.1 DIFFUSION MRI ALTERATION IN THE UNILATERAL 6-OHDA MODEL OF PARKINSONISM (PAPER II)

In paper II, we characterized the changes in brain structure which follow the loss of nigral dopaminergic neurons alongside with associated functional alterations. To do so, diffusion MRI images were obtained post-mortem on 6-OHDA unilaterally lesioned animals. Two approaches were used to analyse the images. The first was to use diffusion tensor imaging together with SPM to detect statistically significant changes on a voxel per voxel basis. Using this approach, it was found that FA decreases in the SNr in the 6-OHDA lesioned hemisphere while radial diffusivity increases in SNc of the same hemisphere. We interpret these changes as being the result of the loss of dendrites of dopaminergic neurons projecting to SNr in a dorsal to ventral direction in parallel of each other.

As a second approach, we used connectometry analysis to identify white matter tracts with altered diffusion properties in the 6-OHDA lesioned animals and we found that the isotropic value of diffusion orientation density function was increased along nigrostriatal tracts of the lesioned hemisphere. We believe that it is related to the loss of dopaminergic axons which remove barriers for protons to diffuse orthogonal to the main direction of the white matter tract.

Both of these findings demonstrated that alterations in diffusion MRI could be detected after 6-OHDA lesioning. The findings from the first approach were aligned with clinical findings (Cochrane and Ebmeier, 2013; Schwarz et al., 2013) as well as an animal study in another model (Boska et al., 2007).

4.2 CHARACTERIZATION AND TRANSLATIONAL VALIDATION OF RS-FMRI NETWORKS IN RATS (PAPER I)

With paper I, the goal was to characterize resting-state networks obtained using rs-fMRI and seed-based correlation analysis and validate their translational value by comparing them to those of humans. By acquiring rs-fMRI datasets in wild type naive rats and healthy humans and using seed-based correlation analysis with seeds bilaterally in the dorsal Str, the ventral Str (the nucleus accumbens), the motor cortex and the posterior cingulate cortex in both species, we could obtain correlation maps with high correlation values in areas of their respective resting-state networks.

The experiments demonstrated that the networks observed using these methods were conserved between the two species despite the fact that rats were anesthetised while people were not.

Confirming the conservation of resting-state networks between the two different species at an healthy state is important in order to study any neurological disease model, as the absence of conserved functional networks would invalidate rs-fMRI and fMRI overall as a valid

approach to study these models. In the contrary this study confirmed the translational value of rs-fMRI as an approach to study animal models of neurological disorders.

4.3 CORTICOSTRIATAL FC AND LOSS OF DOPAMINE (PAPER II, III, IV)

Loss of dopaminergic innervation and lowered tonic level of dopamine in Str are two of the most remarkable hallmarks of PD, dorsal Str, the region equivalent to caudate and putamen, is thus the primary area of interest to characterize the functional alterations in PD. By placing a seed in dorsal Str in paper II, we could observe an increase of FC between the dorsal Str in the lesioned hemisphere and the sensorimotor cortex in unilaterally 6-OHDA lesioned animals when compared to their healthy counterparts. This change was reversible using L-DOPA. In paper III, we used the same approach on rats which were injected with the a-syn AAV. Contrary to the previous experiment, a decrease of FC between dorsal Str and sensorimotor cortex was observed. A similar change was observed in paper IV with the BSSG model of parkinsonism.

The opposite direction of FC alterations between results of paper II in two independent cohorts and those of papers III and IV is counterintuive. However, one must consider the amount of dopaminergic loss in the three models to interpret these results. In the 6-OHDA model, the loss of DAT was quantified at about 90 percents, which is in the range of loss observed in this model. When quantifying tyrosine hydroxylase, an enzyme used as a marker of dopaminergic neurons, in Str in paper III only 18 percents of loss was observed at week 12 in animals overexpressing α-syn. In paper IV, striatal TH level was decreased by 33 percents in rats fed with BSSG 25 weeks after the start of the experiment. Though it is usual to observe a lower decrease of tyrosine hydroxylase compared to DAT in a same model due to the retrograde nature of the dopaminergic neurodegeneration in PD and many of its models, we can affirm that the neurodegeneration levels in papers III and IV is much milder than in paper II.

If we combine this result with the fact that tonic dopamine levels in Str affect the excitability of D1 MSNs and D2 MSNs in opposite directions, we may emit the hypothesis that FC between dorsal Str and its cortical afferent regions form a U-shaped curve in function of tonic striatal dopamine level or dopaminergic innervation and that the minimum FC value is for a dopamine level lower than the level of naive animals, and corresponding to a mild parkinsonian phenotype in rats. This could be explained by the fact that at very low or very high dopamine levels, only one of the two populations of MSNs are excited by cortical afferents and therefore highly synchronised with cortical activity while the other population is mostly silent. For levels of dopamine in between, none of the two populations is as excitable, rendering the overall activity measured through BOLD less synchronized to the BOLD signal of its afferent regions.

To confirm this hypothesis further experiments are needed. One could for example use different combinations of agonists and antagonists of D1 and D2 receptors in healthy rats to

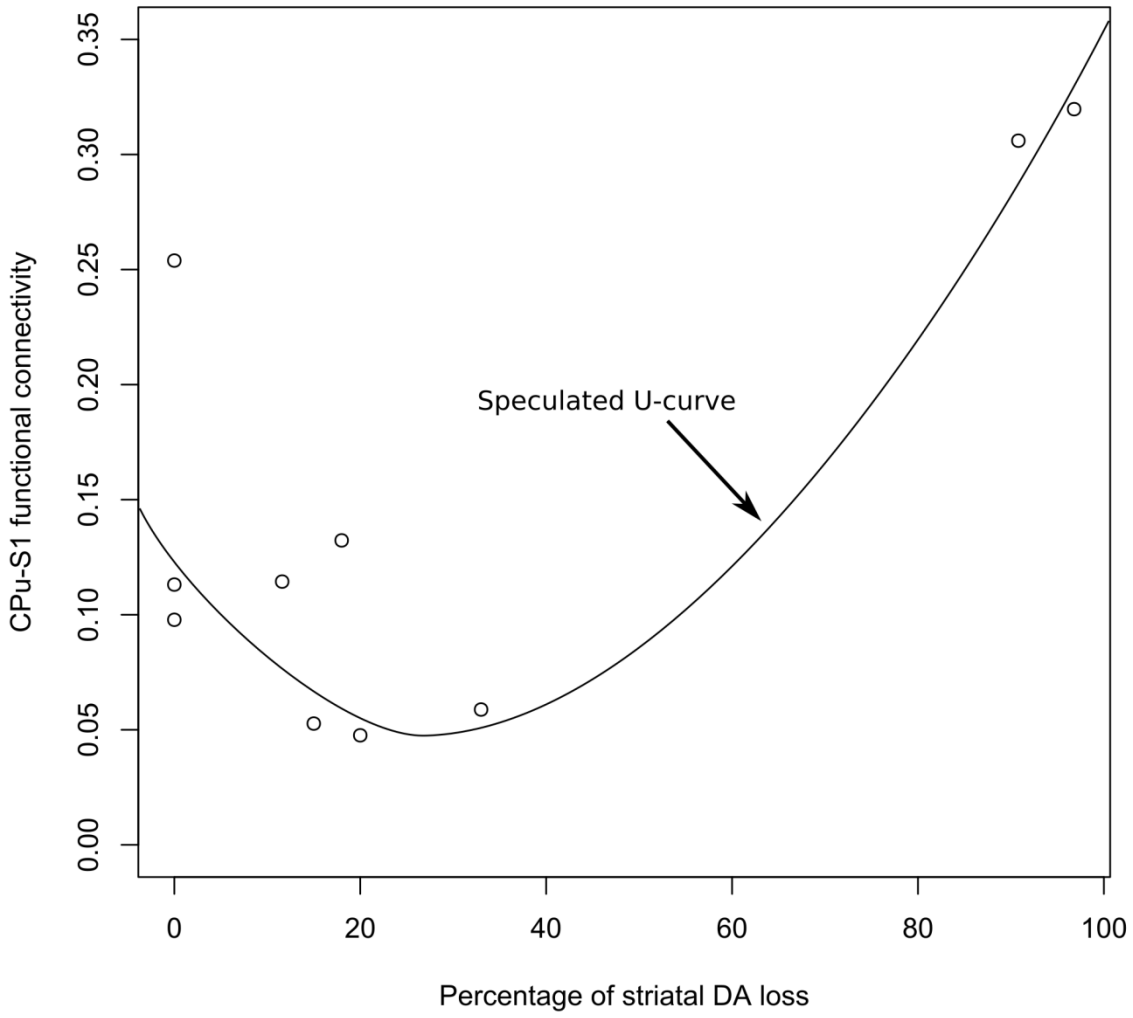

Fig. 4: Measured average functional connectivity between the right Str and the primary somatosensory cortex for different groups from papers II, III and IV in function of striatal dopaminergic innervation loss. A U-shaped curve was overlaid as the potential shape of the function taken by functional connectivity depending on striatal dopaminergic levels.

modulate the activity of D1 MSNs and D2 MSNs and measure corticostriatal FC in those conditions. Also, the existence of such a relation needs to be confirmed in humans in order to prove its translational value, and in particular the location of the minimum relative to the dopamine level must be determined for humans, whether it corresponds to a normal healthy level, or a mild loss of striatal dopamine.

4.4 FC BETWEEN SOMATOSENSORY CORTEX AND PREFRONTAL CORTEX IN RELATION TO PD (PAPER III AND IV)

Experiments from paper III demonstrated that alterations in the level of dopamine affect not only sensorimotor related area but also regions related to emotions and cognition and the relation between these two networks. Indeed, in this paper we could demonstrate that FC within the DMN was increased in the a-syn overexpressing animals at week 8 compared to their green fluorescent protein overexpressing counterparts, but also that FC between primary somatosensory cortex and DMN was decreased at the same timepoint. We also showed that at week 12 FC between primary somatosensory cortex and orbital cortex is decreased in a-syn overexpressing animals. Decrease in FC between the orbital cortex and primary somatosensory cortex could also be observed for BSSG fed rats in paper IV at both timepoints.

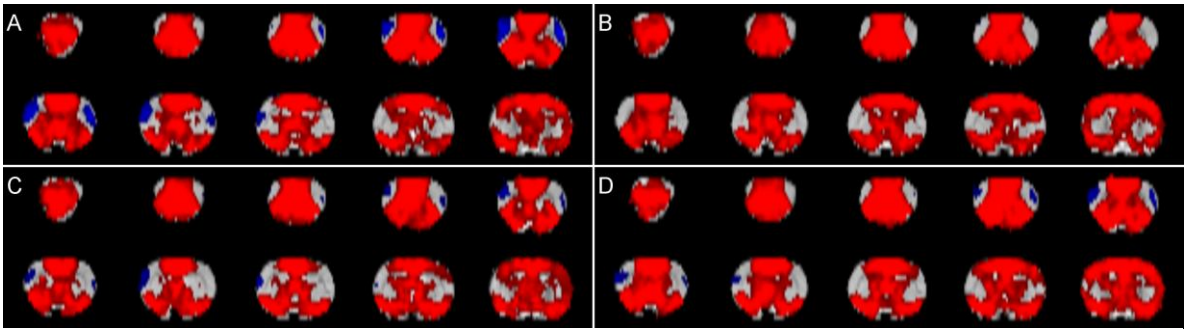

Fig. 5: Results of One-sample t-test for functional connectivity maps with seeds in right orbital cortex for different groups of paper III. A) and C) a-synuclein overexpressing rats at weeks 8 and 12 respectively. B) and D) GFP overexpressing rats at weeks 8 and 12 respectively. p-value < 0.001. Red areas represent positive functional connectivity values and blue areas represent negative values. GFP, green fluorescent protein.

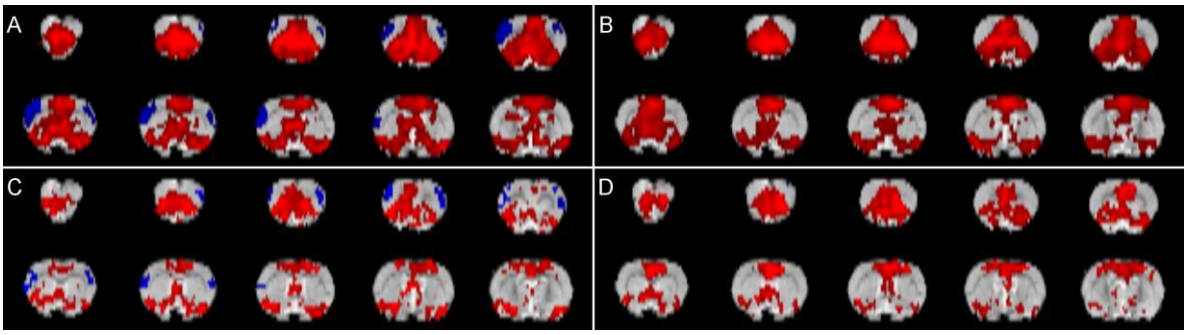

Fig. 6: Results of One-sample t-test for functional connectivity maps with seeds in right orbital cortex for different groups of paper IV. A) and C) Rats fed with BSSG at weeks 18 and 24 respectively. B) and D) Control rats at weeks 18 and 24 respectively. p-value < 0.001. Red areas represent positive functional connectivity values and blue areas represent negative values. BSSG, β-sitosterol-β-D-glucoside.

It is interesting to note that for healthy rats FC between S1 and orbital cortex (and the prefrontal cortex overall) is negative but close to zero, meaning that activity of S1 is slightly anticorrelated with the activity of the prefrontal cortex. One is active when the other is at rest and inversely. This was observed in paper I, but also in papers III and IV. Therefore a

decrease in FC between these areas in parkinsonian animals corresponds to a reinforcement of the anticorrelation observed at a healthy state.

Functional alterations observed involving the prefrontal cortex and in particular the orbital cortex may be related to the development of a depressive phenotype by the animals, which was observed in the a-syn overexpressing model (Caudal et al., 2015). However, there is no evidence of depressive phenotype in the BSSG model that was reported so far.

Another possibility is that these functional alterations are related to cognitive impairments which are frequent in PD, and in particular executive dysfunction. Indeed, the prefrontal cortex is involved in executive function and the corticostriatal circuit formed by caudate nucleus and prefrontal cortex is key to executive function (Leh et al., 2010). However, more needs to be done to show alterations in cognitive function in both the a-syn overexpression and the BSSG models.

5 GENERAL CONCLUSIONS

The results shown within this thesis demonstrate clearly that rs-fMRI has a translational value to study neurological and psychiatric disorders and their models. We also clearly demonstrated that rs-fMRI could be conducted in a reproducible manner and that the findings are consistent between studies.

More specifically, we have shown here that dopaminergic neurodegeneration occuring in models of parkinsonism provoke functional alterations that can be characterized using rs-fMRI. Two main features were demonstrated, an alteration in FC between the dorsal Str and the sensorimotor areas and a reinforcement of negative FC between prefrontal cortex and primary somatosensory cortex in rats presenting a mild parkinsonian pathology. More needs to be done to characterize these and other models of parkinsonism with rs-fMRI and these changes need yet to be demonstrated in clinical studies. A recent meta-analysis on rs-fMRI studies in PD patients seem to support the corticostriatal change observed here but more needs to be done to fully characterize rodents models of parkinsonism with rs-fMRI. Moreover, these changes need yet to be confirmed in clinical studies.

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