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Cerebral reorganization in premotor parkinsonism

Bart F.L. van Nuenen
Cerebral reorganization in premotor parkinsonism

Proefschrift

The studies presented in this thesis were carried out at the Radboud University Nijmegen Medical Centre, Donders Institute for Brain, Cognition and Behaviour, Centre for Neuroscience, Department of Neurology, Nijmegen, the Netherlands, at the Donders Institute for Brain, Cognition and Behaviour, Centre for Cognitive Neuroimaging, Radboud University Nijmegen, Nijmegen, the Netherlands and at the Department of Neurology, Christian-Albrechts University, Kiel, Germany.

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General introduction and aims of this thesis
Introduction

Parkinson’s Disease

Parkinson’s disease (PD) is a neurological disorder which was first described by James Parkinson in 1817. It is nowadays the second most common neurodegenerative disorder, coming second to Alzheimer disease only (de Lau and Breteler, 2006). Clinically, PD has traditionally been defined by the presence of cardinal motor signs: rest tremor, rigidity, bradykinesia and postural instability. The clinically based diagnosis of PD is typically made based upon the presence of these motor features, as well as the absence of atypical findings suggestive of an alternative diagnosis (the so-called ‘red flags’), plus a favorable response to levodopa (Samii et al., 2004). The diagnostic criteria most frequently used in clinical research are those of the UK Parkinson’s Disease Society Brain Bank (Gibb and Lees, 1988). Differentiating PD from other parkinsonian disorders such as progressive supranuclear palsy (PSP) or multiple system atrophy (MSA) can be challenging, particularly in the early course of the disease. Both standard neuroimaging (Meijer et al., 2011) as well as advanced neuroimaging techniques (Brooks, 2010b, a) have been used to facilitate a more accurate early diagnosis of PD, but are currently not widely used in clinical practice (Brooks, 2010a).

The diagnosis PD is therefore made on clinical grounds, and ‘definite’ PD cannot be diagnosed during life but requires neuropathological confirmation. The pathological hallmarks of the disease include dopaminergic cell loss within the substantia nigra pars compacta and the presence of Lewy bodies (LBs) and Lewy neurites (collectively referred to as Lewy-related pathology) in vulnerable populations of neurons (Braak et al., 2003, Braak et al., 2004).

To investigate the neurobiological changes occurring in PD, research initially relied mainly on post-mortem material from PD patients (Bernheimer et al., 1973, Kish et al., 1988, Braak et al., 2003) and on animal models of PD (Langston et al., 1984). These studies have been valuable for defining the neuropathology of PD. However, post-mortem material is collected mostly from patients with advanced disease, where brain pathology is obscured by secondary changes and perhaps by age-related rather than PD-related changes. Moreover, pathological studies cannot address functional changes in the PD brain that occur prior to neuronal death. Furthermore, animal models are not able to fully capture all aspects of human PD, such as the changes within the cognitive domain (Di Monte, 2003). For example, the most commonly used animal model is based on chronic exposure to 1-methyl-
CHAPTER 1
GENERAL INTRODUCTION AND AIMS OF THIS THESIS

compensatory mechanisms serving to delay or even reduce clinical symptoms (Bezard and Gross, 1998). In early stage PD, fMRI has provided evidence for such cerebral reorganization (Helmich, 2011). However, little is known about the premotor phase of parkinsonism in humans. The premotor phase of the disease has thus far mainly been studied in primates exposed to MPTP. In this model, low doses of MPTP were repeatedly administered over a time course of 1 month to cause progressive striatal dopaminergic denervation, but without causing clinically

4-phenyl-1,2,5,6-tetrahydropyridine (MPTP), a neurotoxin that damages the nigrostriatal dopamine tract (Langston et al., 1984, Bloem et al., 1995, Bloem and Roos, 1995). While nigrostriatal degeneration in PD patients follows a gradual progression, such a time course is much more difficult to achieve using these MPTP injections in animals (Mounayar et al., 2007).

In the last decades, development of brain imaging methods such as functional magnetic resonance imaging (fMRI; see BOX1) has enabled non-invasive assessments of brain activity in vivo. These neuroimaging tools can be used to investigate functional brain changes occurring in patients with clinically overt PD. Functional cerebral reorganization in such symptomatic phases of PD can take different forms. For example, it might be localized to specific brain regions, or involve system-level changes at the network level. Furthermore, functional reorganization in PD may have different behavioural consequences. That is, they could reflect pathological alterations that produce clinical or behavioural impairments, but could also represent

Box 1.1 Functional magnetic resonance imaging (fMRI).

Functional magnetic resonance imaging (fMRI) is a method that uses MRI to investigate which areas of the brain are active during performance of a specific task (figure 1.1). It was developed in the early 1990s (Ogawa et al., 1990, Kwong et al., 1992) and since then has grown to become the dominant technique in cognitive neuroimaging. An advantage of fMRI is that it allows for noninvasive recording of brain signals, without for example the risks of exposure to radiation that is associated with some other neuroimaging techniques such as X-ray computed tomography scans. In addition, it allows for recording with a high spatial resolution of 3-6 mm. A disadvantage is that the temporal resolution is relatively low compared to techniques such as electroencephalography (EEG). This is related to the fact that fMRI does not measure neuronal activity directly. Rather it measures the changes in blood flow and blood oxygenation in the brain related to the neural activity in the brain. Functional MRI cannot detect absolute activity of brain regions, it can only detect differences in brain activity between different conditions. During the fMRI experiment, the subject is therefore asked to perform several tasks. Each of these conditions is repeated several times and separated by rest periods. It is important that the experimental and control conditions are as similar as possible. If the conditions differ in more than one way, there could be multiple explanations for the differences in cerebral activity.

Figure 1.1
A. Photograph of a MRI scanner, the main magnetic field is 3 Tesla. Subjects lay supine in the scanner. Task stimuli are programmed on a computer in the control room, and presented onto the screen via a beamer. Subjects can respond in different ways (e.g. button press with hands or feet, or eye-movements with an eye-tracker). B. An image of the brain with areas of statistically significant areas when a healthy subject is asked to prepare to grasp and lift a weight.
discernable symptoms in these animals (Bezard et al., 2001). This “subacute” MPTP model revealed that progressive striatal dopamine depletion can trigger several compensatory mechanisms, both within and outside the basal ganglia (Bezard et al., 2003).

Other insights in the premotor phase from PD in humans come from epidemiologic studies, in particular retrospective case control series, and also large prospective cohort studies. These studies have suggested that certain non-motor manifestations, such as rapid eye movement sleep behavior disorder (Uchiyama et al., 1995; Schenck et al., 1996; Siasny-Kolster et al., 2006), anxiety disorders (Shiba et al., 2000; Weisskopf et al., 2003; Bower et al., 2010), hyposmia (Ponsen et al., 2004, Ponsen et al., 2009, Ponsen et al., 2010, Berendse et al., 2011), constipation (Abbott et al., 2001; Savica et al., 2000a) and anemia (Savica et al., 2009b), may precede the motor manifestations of PD by a long time. This indicates that there is a premotor period, which might be as long as 20 years before the onset of motor signs, although it remains very difficult to make reliable estimates about the duration of this premotor phase. (Note that it is presumably better to use the term premotor phase, rather than preclinical phase, because it can be argued that the presence of non-motor symptoms in the years before onset of motor symptoms is in fact already a clinical phase, even though it is currently not yet defined as such. In this thesis, we will consistently refer to this period as the premotor phase). It is conceivable that cerebral compensatory processes are at least partially responsible for the absence of the characteristic motor symptoms, despite the presence of PD-related pathology (as can be identified in vivo using nuclear imaging of the dopaminergic system in these individuals during the premotor phase). However, it remains unknown just how the human motor system adapts to this slowly progressive nigrostriatal dopaminergic denervation; one of the reasons is the fact that we have no reliable diagnostic test at hand that can be easily applied to the general population, in order to identify individuals that are in the premotor stage of PD. Fortunately, new opportunities to study the premotor phase of PD have arisen in the past decade, due to advances in the field of genetics. Specifically, as we will point out in more detail below, there are now possibilities to study asymptomatic carriers of mutations in different genes that have been shown to cause genetic forms of PD.

The genetics of parkinsonism

In the past decade, genetic studies in PD families from different geographical regions worldwide have confirmed the hypothesis that PD has a substantial genetic component. Since the first locus (PARK1; SNCA) (Polymeropoulos et al., 1996, Polymeropoulos et al., 1997) was described in 1996, a total of now 18 different loci has been identified through traditional cloning approaches, linkage studies or genome-wide association studies (Gasser et al., 1998; Leroy et al., 1998; Hicks et al., 2002; Pankratz et al., 2003, Strauss et al., 2005, Belin and Westerlund, 2008, Lautier et al., 2008, Di Fonzo et al., 2009, Paisan-Ruiz et al., 2009, Pankratz et al., 2009, Satake et al., 2009, Simon-Sanchez et al., 2009, Hamza et al., 2010). Mutations in eight of the corresponding genes (SNCA, LRKK2, Parkin, DJ1, PINK1, ATP13A2, VPS35 and EIF4G1) have conclusively been demonstrated to cause monogenetic forms of parkinsonism (Lesage and Brice, 2009, Chartier-Harlin et al., 2011, Vilarino-Guell et al., 2011). These rare genetic forms of PD show a substantial clinical phenotypic overlap with sporadic, non-genetic PD. Non-manifesting individuals who carry a single heterozygous mutation in the Parkin (PARK2) or PINK1 (PARK6) genes - both of which associated with recessively inherited PD in case of two pathogenic mutations - have attracted particular interest. In these individuals, positron emission tomography (PET) – that has been used to evaluate dopaminergic neurotransmission – has shown a mild presynaptic dopaminergic dysfunction despite absence of corresponding clinical manifestations (Hilker et al., 2001, Khan et al., 2002a, Khan et al., 2002b). In addition, heterozygous mutation carriers have an increased risk to develop signs of PD throughout their life (Hedrich et al., 2002, Hedrich et al., 2006). Therefore, it has been argued that non-manifesting carriers of a single mutant allele in these two recessive PD genes provide a human model to study downstream changes within the cortico-basal ganglia-thalamo-cortical circuitry in the context of a subclinical loss of dopamine-producing cells in the substantia nigra (van Elmeren and Siebner, 2006).

In healthy subjects with or without such mutations that render them to be at risk for PD, it is now possible to study cerebral activity (using techniques such as fMRI) to identify primary, premotor functional changes. The identification of these (compensatory) mechanisms is important to expand our knowledge about the neurobiological mechanisms that are at play in this premotor phase of PD. In the future, this may lead to new therapies aimed at modulating functional reorganization in vivo, for example by tuning down pathological changes or by facilitating compensatory mechanisms using transcranial magnetic stimulation (TMS). For that purpose we also conducted studies, as outlined in the second part of this thesis, aimed to induce temporary inhibition of different cortical areas with TMS (see BOX2) to test...
whether it is possible to capture cerebral reorganization by combining TMS and fMRI in healthy controls and PD patients.

**Box 1.2 Transcranial magnetic stimulation (TMS).**

In 1985, Barker et al. (Barker et al., 1985) developed a method of external brain stimulation, namely transcranial magnetic stimulation (TMS). This is a noninvasive method to cause depolarization or hyperpolarization in the neurons of the brain. The scientific principle on which magnetic stimulation is based was discovered by Michael Faraday in 1831. He described the phenomenon of mutual induction, whereby current flows in a secondary circuit when it is brought near a current-carrying primary circuit. In 1896, D’Arsonval reported that flickers of light were seen by volunteers when their heads were placed in a time-varying magnetic field. In 1965, Blickford and Freming demonstrated muscular contractions in animals and humans after magnetic stimulation. Twenty years later, in 1985, Barker (figure 1.2) Stimulated the human brain with a magnetic stimulator as it is being used nowadays. For TMS, we use an electromagnetic coil that is held over the subject’s head. When current passes through the coil, it generates a magnetic field that can penetrate the subjects’ scalp and skull. By rapidly changing the magnetic field, weak electric currents can be induced in the nearby brain tissue (electromagnetic induction). This allows for triggering of brain activity with minimal discomfort. The effects of TMS can be divided into two types depending on the mode of stimulation. First, single or paired pulse TMS causes neurons in the cortex under the site of stimulation to depolarize and discharge an action potential. When TMS is applied over the primary motor cortex, it produces a response that can be seen directly, in the form of muscle twitches. The response results from the activation of corticospinal neurons in the motor cortex that project directly to the spinal cord and that are connected in the spinal cord with alpha motor neurons that activate the muscles. The motor response induced by TMS is called the motor-evoked potential (MEP; see figure 1.3) which can be recorded with electromyography. The amplitude of the MEP reveals the net excitability of the corticospinal system (Rothwell et al., 1991). If used on the occipital cortex, ‘phosphenes’ (flashes of light) might be perceived by the subject. In most other areas of the cortex, the participant does not consciously experience any effect, but his or her behaviour may be slightly altered (e.g. slower reaction time on a cognitive task), or changes in brain activity may be detected using sensing equipment such as EEG (Pascual-Leone et al., 2002). Second, repetitive TMS (rTMS) produces longer-lasting effects that persist after the actual stimulation has stopped.
May prevent or delay clinical manifestation. In addition to mutations causing monogenic forms of PD, common polymorphisms in genes that influence mono-aminergic signalling or synaptic plasticity may have modifying effects on distinct aspects of PD. I also discuss how functional and structural neuroimaging can be used to better characterize genotype-phenotype correlations. Furthermore, in chapter 3, I reviewed how fMRI can be used to identify compensatory mechanisms that help to prevent development of overt disease.

In chapter 4, I used a combined neurogenetic-neuroimaging approach to examine the functional consequences of premotor dopaminergic nigrostriatal dysfunction in the human motor system. Specifically, I examined how a single heterozygous mutation in two genes associated with recessively inherited PD (Parkin and PINK1) alters the cortical control of sequential finger movements during fMRI. The most important question which I address is whether the observed cerebral reorganization reflects a “generic” or “gene-specific” compensatory mechanism to maintain motor function in the context of a mild dopaminergic deficit. For this purpose, we studied subjects with mutations in different PD genes and compared the nature of their adaptive plasticity. In chapter 5, I extended this work by examining premotor cerebral reorganization in non-manifesting mutation carriers of one of the PD genes and compared the nature of their adaptive plasticity with that observed in healthy controls and patients with clinically overt PD.
I provide a method, in which I transiently inhibited the left PMd and right LRRK2 of this thesis, I used TMS to transiently disrupt neuronal gene, described in these behavioral changes with fMRI. This led to the last study in PD patients, we can indeed induce behavioral changes with TMS and also to map and correlate condition effects of cTBS on preparatory brain activity were assessed with fMRI, cortex (PMd) by using continuous theta burst stimulation (cTBS) in controls and PD patients. Specifically, I processing in cortical areas, in order to test their functional relevance in healthy controls and overt PD patients. Finally, in chapter 8 I provide a summary and general discussion of my findings and sketch several future perspectives.

References


CHAPTER 1

GENERAL INTRODUCTION AND AIMS OF THIS THESIS


Identification of compensation in premotor parkinsonism
Imaging the impact of genes on Parkinson’s disease

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CHAPTER 2

IMAGING THE IMPACT OF GENES ON PARKINSON’S DISEASE

Abstract

Although Parkinson’s disease (PD) has traditionally been considered to be a non-genetic disorder, recent progress in the neurogenetics of PD provided converging evidence that genetic factors play a relevant role in the aetiology of PD. The strongest case for a genetic contribution to PD was made by the discovery of mutations in single genes that can cause autosomal dominant (SNCA and LRRK2 gene) or recessive (Parkin, PINK1, DJ-1, and ATP13A2 gene) forms of PD. Here, we review how structural and functional neuroimaging of individuals carrying a mutation in one of the PD genes have offered a unique avenue to obtain new insights into the pathogenesis of PD. In symptomatic mutation carriers (i.e. those with overt disease), brain mapping can help to link the molecular pathogenesis of PD more directly with functional and structural changes in the intact human brain. In addition, neuroimaging of presymptomatic (i.e. non-manifesting) mutation carriers has emerged as a valuable tool to identify mechanisms of adaptive motor reorganization at the preclinical stage that may prevent or delay clinical manifestation. In addition to mutations causing monogenic forms of PD, common polymorphisms in genes that influence monoaminergic signalling or synaptic plasticity may have modifying effects on distinct aspects of PD. We also discuss how functional and structural neuroimaging can be used to better characterize these genotype-phenotype correlations.

Introduction

Parkinson’s disease (PD) is the second most common neurodegenerative disorder (de Lau and Breteler, 2006). The traditional view dictates that affected patients present with a variable combination of motor symptoms, including bradykinesia, rigidity, resting tremor and – in later stages of the disease – also with postural instability. However, it is becoming increasingly clear that these “traditional” motor impairments represent just the tip of the iceberg, and that these are typically accompanied (or indeed, even preceded) by a variety of non-motor symptoms, including hyposmia, cognitive decline, mood disorders, pain, autonomic dysfunction and sleep disorders (Langston, 2006). In this review, we will focus on the pathophysiology of the motor impairments in PD, and how these are linked to the underlying genetic abnormalities that can be found in subgroups of patients with parkinsonism. We have limited ourselves to the motor manifestations of PD as they represent the best “accessible” outcome measures in imaging and genetic studies.

The motor impairments in PD result mainly from progressive neuronal loss of dopaminergic neurons in the substantia nigra pars compacta and can improve markedly with dopaminergic drugs (Gibb, 1991, Gibb and Lees, 1991, Damier et al., 1999). Interestingly, the motor system has a substantial potential to cope with the slowly progressive degeneration of the nigrostriatal dopaminergic projections. Clinical symptoms only emerge when around 70-80% of nigrostriatal nerve terminals have undergone degeneration (Bernheimer et al., 1973). The logical implication is that for most patients, the neurodegenerative process has likely started well in advance of the first overt clinical motor symptoms, although the length of this presymptomatic period remains unknown. Indeed, post-mortem histological examinations have identified individuals who show Lewy neurites and Lewy bodies (the histological hallmarks of PD) in various brainstem areas but who had never developed parkinsonism during life time (Braak et al., 2003, Braak et al., 2004).

It has been a widely held notion that PD is mainly caused by environmental factors, with genetic factors playing only a very minor role in the pathogenesis. This “environmental” theory was supported in particular by the identification of “selective” nigrostriatal neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which can cause parkinsonian symptoms with a remarkable resemblance to idiopathic PD in rodents, primates and even in humans (Langston et al., 1983, Bloem and Roos, 1995). However, the ideas about the aetiology of PD have changed considerably in the last decades. Advances in neurogenetics have provided strong evidence that what clinicians perceive as “idiopathic PD” can in fact be genetically determined. There are converging sources of supporting evidence (Martin et al., 1973, Tanner et al., 1999, Warner and Schapira, 2003), but the case is certainly
strongest for monogenic forms of familial PD that are caused by mutations in specific genes (Gasser, 2007; Klein and Schlossmacher, 2007). Although monogenic forms account for only a small fraction of PD patients (perhaps around 3 to 5% of all cases), they have considerably stimulated the field of PD research. For example, the cellular pathways that are affected by monogenic variants of PD have provided important clues regarding the molecular pathogenesis in typical sporadic PD (Gasser, 2007). In this review, we will focus on another important research area that greatly profited from the advances in PD genetics, namely the field of neuroimaging. Specifically, we evaluate the structural and functional brain mapping studies that have been performed in individuals carrying a mutation in a specific PD gene, and discuss how this “neurogenetics-neuroimaging approach” provides unique means to tap into important pathophysiological aspects of PD.

**Monogenic forms of Parkinson’s disease**

The genes and chromosomal loci linked to familial forms of PD have been designated as PARK1–13. These loci include six autosomal dominant (PARK1 (=4), 3, 5, 8, 11 and 13), four recessive (PARK2, 6, 7, and 9), one X-linked (PARK12), and one form with an as yet unknown mode of transmission (PARK10). Mutations in the LRRK2, Parkin and PINK1 genes are the clinically most relevant types because they are relatively frequent and their clinical phenotype shows substantial overlap with that of sporadic (non-familial) PD. A detailed description of the genetics of PD is beyond the scope of this paper and has been covered in several recent reviews (Shadrina and Slominskii, 2006; Gasser, 2007; Klein and Lohmann-Hedrich, 2007; Klein et al., 2007; Klein and Schlossmacher, 2007; Tan and Skipper, 2007; Belin and Westerlund, 2008). Here we will rather focus on a short description of well-established forms of genetic PD for which neuroimaging data have become available, i.e. PARK1(=4), PARK2, PARK6, PARK7 and PARK8. In the following sections, these monogenic forms of parkinsonism will be grouped according to their mode of inheritance. This is in agreement with functional findings that suggest a gain-of-function mechanism for dominant and a loss-of-function mechanism for recessive forms. However, things appear to be more complex as penetrance (percentage of mutation carriers that actually develop the disease) is remarkably reduced in dominant forms. Conversely, a putative role of single heterozygous mutations as a susceptibility factor has been suggested in recessive forms (Klein et al., 2007).

**PARK1(=4) (dominant)**

In 1997, the alpha-synuclein (SNCA) gene was the first one to be unequivocally associated with familial parkinsonism (Polymeropoulos et al., 1997). In addition to the three point mutations, a number of families with parkinsonism have been identified that carry single allele triplications (initially assigned as PARK4) or duplications of the wild-type SNCA gene (Farrer, 2006). Penetrance has been described to be as low as 33% (Nishioka et al., 2006), which is highly relevant for this review as it implies that many carriers of a pathogenic genetic abnormality somehow “manage” to remain asymptomatic. Why a substantial proportion of carriers never goes on to develop overt parkinsonism remains unknown. Here neuroimaging may provide some of the answers and help to clarify the pathophysiological substrate for this variable penetrance. For many of the SNCA-linked cases, the severity of the phenotype appears to depend on gene dosage, and patients with SNCA duplications clinically resemble ‘idiopathic’ PD patients more than those with triplications, although the phenotypic spectrum can be remarkably broad (Fuchs et al., 2007). SNCA is abundantly expressed in the vertebrate nervous system, where it is believed to participate in the maturation of presynaptic vesicles and to function as a negative co-regulator of neurotransmitter release (Vekrellis et al., 2004). SNCA has a propensity to aggregate owing to its hydrophobic non-amyloid-beta domain. Intriguingly, oligomer-forming species of SNCA, along with truncated, oxidized and phosphorylated variants have been found in insoluble inclusions (‘Lewy bodies and Lewy neurites’) of the human brain including SNCA-linked cases (Spillantini et al., 1997).

**PARK8 (dominant): LRRK2**

The LRRK2 (leucine-rich repeat kinase 2) gene has been identified by two independent groups (Paisan-Ruiz et al., 2004; Zimprich et al., 2004) and is now recognized as the most common known cause of familial and sporadic parkinsonism. Mutations in the LRRK2 gene are mostly associated with late-onset, classical parkinsonism. LRRK2 is a large gene that consists of 51 exons encoding a 2527-amino acid protein named LRRK2 featuring several functional domains. To date, more than 50 variants have been reported in this gene. By far the most frequent and best studied mutation is the c.6055G>A (p.G2019S) substitution that accounts for ~1.5% of all index cases with late-onset, classical parkinsonism (Healy et al., 2008). As for PARK1, penetrance is incomplete and is age-dependent, reaching perhaps around 70% depending on the age of the mutation carrier. The high prevalence of pathogenic mutations in the LRRK2 gene is incomplete and also age-dependent, ranging perhaps around 70% depending on the age of the mutation carrier. The high prevalence of pathogenic mutations in the LRRK2 gene also implies that there are many presymptomatic individuals, with the same implications for neuroimaging as for PARK1. There is a surprisingly high degree of neuropathological heterogeneity even in members of the same family carrying an identical mutation, ranging from Lewy body-positive parkinsonism, to diffuse Lewy body disease, to nigral degeneration without distinctive histopathology, and to progressive supranuclear palsy-like pathology (Wszolek et al., 2004). LRRK2 functions as a protein kinase, mutations of which alter its phosphorylation activity through a proposed gain-of-
function mechanism (Lu and Tan, 2008). Unexpectedly, the expression rate of the \textit{LRRK2} gene (as compared, for example, to the \textit{Parkin} or \textit{DJ-1} gene) in mammalian brain is low in the predominantly affected dopaminergic neurons of the human substantia nigra, whereas high expression rates of \textit{LRRK2} were found in striatal neurons that receive dopaminergic input (Galter et al., 2006).

**PARK2 (recessive): Parkin**

Overall, \textit{Parkin} mutation carriers tend to have an earlier age at disease onset, a slower disease progression and often feature a better response to levodopa than patients without \textit{Parkin} mutations (Lohmann et al., 2003), as do carriers of mutations in the \textit{PINK1} and \textit{DJ-1} genes (see below). Mutations in the \textit{Parkin} gene (Kitada et al., 1998) represent the most common known factor responsible for early-onset parkinsonism (10–20%), and have been found across all tested ethnic groups (Hedrich et al., 2004). One might predict that this recessively inherited disorder should spare heterozygous mutation carriers; yet it appears that carrying a single mutant allele acts as a susceptibility factor, increasing the risk of developing overt parkinsonism at a later age (Klein et al., 2007). The obvious implication for this review on neuroimaging is that carriers of single heterozygous mutations may represent another model – complementary to the presymptomatic dominant mutation carriers – of presymptomatic parkinsonism. Similar considerations apply to the other recessive forms of parkinsonism discussed below (PARK6 and PARK7).

The gene product, called Parkin, is an E3-type ubiquitin ligase that is involved in the proteasomal degradation of target proteins (Shimura et al., 2000). The available E3 activity of many (but not all) PD-linked mutants is disrupted in \textit{ex vivo} experiments; others affect the solubility, localization and binding properties of Parkin. Indeed, reduced ubiquitin ligase activity may only be one of several pathogenetic mechanisms (Feany and Pallanck, 2003) as recent studies have delineated an essential role of Parkin in mitochondrial integrity (Deng et al., 2008). Only two of the six \textit{Parkin} mutant PD brains that have come to autopsy showed typical Lewy bodies, while the other four did not (reviewed in Pramstaller Ann Neurol 2005 (Pramstaller et al., 2005)).

**PARK6 (recessive): PINK1**

Two homozygous mutations in the \textit{PINK1} (\textit{PTEN-induced kinase 1}) gene were initially described in three families with autosomal recessive, early-onset parkinsonism (Valente et al., 2004). The frequency of \textit{PINK1} mutations ranges from 1–8% in patients of different ethnicities (often selected for their young age of onset and positive family history). Most of the currently described mutations are localized within the functional serine/threonine kinase domain of PINK1. Wild-type PINK1 protein is localized inside mitochondria and has been demonstrated to have a pro-mitochondrial function. In addition, all known PINK1 interactors, TRAP1, HSP90 and

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**Table 1**

Synopsis of well established forms of genetic PD.

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Mode of inheritance</th>
<th>Locus</th>
<th>Gene/protein</th>
<th>Main clinical features</th>
<th>OMIM #</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARK1/ PARK4</td>
<td>Autosomal dominant</td>
<td>4q21-q23</td>
<td>SNCA (alpha-Synuclein)</td>
<td>Early-onset parkinsonism (~40 years), dementia, reduced life span</td>
<td>168601</td>
</tr>
<tr>
<td>PARK8</td>
<td>Autosomal dominant</td>
<td>10q12</td>
<td>LRRK2 (Leucine-rich repeat kinase 2)</td>
<td>Classical parkinsonism</td>
<td>607060</td>
</tr>
<tr>
<td>PARK2</td>
<td>Autosomal recessive</td>
<td>6q25-q27</td>
<td>PARK2</td>
<td>Early onset (~30-40 years), responsive to L-dopa</td>
<td>600116</td>
</tr>
<tr>
<td>PARK6</td>
<td>Autosomal recessive</td>
<td>1p35-p36</td>
<td>PINK1</td>
<td>Early onset (~30-40 years), responsive to L-dopa, frequently psychiatric features</td>
<td>605909</td>
</tr>
<tr>
<td>PARK7</td>
<td>Autosomal recessive</td>
<td>1p36</td>
<td>DJ-1</td>
<td>Early onset (~30-40 years), rarely juvenile</td>
<td>606324</td>
</tr>
</tbody>
</table>

OMIM = Online Mendelian Inheritance in Man.
Omi/HtrA2 have a chaperone activity, suggesting that PINK1 might participate in the detoxification of proteins through these interactions (for review, see Plun-Favreau PNAS 2008 (Plun-Favreau and Hardy, 2008)).

PARK7 (recessive): DJ-1
The DJ-1 gene (Bonifati et al., 2003) is associated with early-onset parkinsonism in about 1–2% of cases. The DJ-1 gene is ubiquitously expressed and was initially described in association with oncogenesis and male rat infertility. DJ-1 has been shown in mice to co-regulate the D2 dopamine receptor signalling (Goldberg et al., 2005). The protein has also been found to confer chaperone-like activity, and several reports convincingly demonstrated that DJ-1 acts as an intracellular protector against oxidative stress (Dodson and Guo, 2007). Furthermore, mitochondrial localization of DJ-1 leads to enhanced neuroprotection (Junn et al., 2009), implicating also this protein in mitochondrial function.

These forms of monogenic parkinsonism create a unique perspective for studies that use neuroimaging to understand the pathophysiology of parkinsonism. This is based on the following notions: i) Genetically defined parkinsonism represents a more homogeneous and etiologically defined entity as compared to ‘idiopathic’ PD. This homogeneity helps to reduce variability, which is one of the prime challenges in neuroimaging studies. ii) Neuroimaging yields important insights into the pathophysiology of the various genetic forms; conversely, specific features of genetic forms, such as pattern and type of neurodegeneration, areas of highest gene expression or protein function, may inform the choice of neuroimaging techniques and paradigms. iii) Neuroimaging studies of non-manifesting PD mutation carriers and of asymptomatic mutation carriers with only subtle motor signs suggestive of parkinsonism open up a window for the investigation of preclinical and very early disease phases in vivo, and for the possible identification of compensatory mechanisms that help to prevent development of overt disease. An additional advantage of truly non-manifesting carriers is that these subjects can be tested functionally (i.e. by asking them to perform a particular motor task in the scanner), without concerns that differences in performance (caused by disease signs) by themselves change cerebral activation and thereby obscure interpretation of the data.

Here we review the synergistic applications of neuroimaging and genetics of PD. From a conceptual point of view, we will distinguish studies done in non-manifesting carriers from those done in subjects with clinically overt disease. While we mainly focus on neuroimaging of monogenic forms of PD, we will also discuss how neuroimaging may help to assess the influence of common genetic variation/polymorphisms on distinct aspects of PD.

Mapping striatal dopaminergic neurotransmission
After mutations in specific genes that can cause familial forms of PD had been identified, an obvious question was whether these mutations would affect striatal dopaminergic neurotransmission in a similar fashion as in the common sporadic form of PD. Specifically, symptomatic mutation carriers were examined with radiotracer imaging techniques such as Positron Emission Tomography (PET) or Single Photon Emission Computed Tomography (SPECT). Various techniques have been used for this purpose (see e.g. (Piccini, 2004, Nandhagopal et al., 2008)), aiming to test either presynaptic or postsynaptic aspects of nigrostriatal integrity (Table 2).

Symptomatic patients with the sporadic form of PD show a reduction in striatal [18F]FDOPA uptake, as well as a marked reduction in striatal VMA2 and DAT binding (Brooks, 2004, Shih et al., 2006a). Signal decreases in the striatum typically show a rostro-caudal gradient, the putamen being more affected than the caudate nucleus, and with a stronger dysfunction in the hemisphere contralateral to the clinically most affected side (Nandhagopal et al., 2008). Postsynaptically, early-stage PD patients may display an increase in striatal D2 receptor binding which normalizes with chronic dopaminergic replacement therapy (Piccini, 2004, Verstappen et al., 2007).

Radiotracer imaging of manifesting mutation carriers
We will first discuss the radiotracer imaging studies done in subjects with clinically overt disease (symptomatic mutation carriers). Table 3 lists the published PET and SPECT studies of patients with monogenic forms of PD. All studies that have used [18F]FDOPA PET in symptomatic mutation carriers revealed a marked reduction of pre-synaptic [18F]FDOPA uptake, similar to age-matched patients with sporadic non-hereditary PD. The same pattern emerged with PET or SPECT of DAT, showing a consistent bilateral reduction in striatal DAT. Similar to sporadic PD, most studies also found a caudo-rostral gradient with a stronger impairment of pre-synaptic dopaminergic function in putamen compared to the caudate nucleus. However, in contrast to sporadic PD, the pre-synaptic changes in putaminal dopaminergic neurotransmission tend to display a more symmetrical pattern in Parkin and PINK1 mutation carriers, along with additional reductions in caudate and midbrain (Khan et al., 2002a, Scherfler et al., 2004, Varrone et al., 2004, Hu et al., 2006). Repeated [18F]FDOPA PET imaging revealed a slower progression rate in presynaptic dysfunction in manifesting parkin mutation carriers relative to patients with common sporadic PD (Khan et al., 2002a, Pellecchia et al., 2007), consistent with the clinical disease course which tends to be more benign in recessive parkinsonism. Post-synaptic dopamine function was found to be normal with [12C]Raclopride PET in patients with mutations in the SNCA gene (PARK1, n=4), the PINK1 gene (PARK6, 2005).
were on long-term treatment with dopaminergic drugs (Khan et al., 2002b, Scherfler et al., 2004, Varrone et al., 2004, Hu et al., 2006). It is unclear if this finding is caused by the disease itself or by the medication. A recent [11C] Raclopride PET study showed an up-regulation of striatal D2-receptors in dopaminergic drug-naïve patients with a parkin mutation, in a similar fashion as drug-naïve patients with sporadic PD. Patients with sporadic PD show a normalisation of striatal [11C] Raclopride binding after long-term treatment with dopaminergic drugs. This makes it more likely that the decrease in striatal [11C] Raclopride binding in levodopa treated patients with a parkin mutation is caused by a greater susceptibility to the exposure to dopaminergic medication rather than primarily by the genetic defect itself, compared to patients with sporadic PD (Scherfler et al., 2006).

Taken together, the PET findings in patients with monogenic forms of PD confirm a substantial loss of presynaptic dopaminergic nigrostriatal afferents without accompanying post-synaptic neurodegeneration. Except for a few subtle differences and the decrease in striatal [11C] Raclopride binding in patients with a parkin mutation, which can possibly be explained by a higher susceptibility to dopaminergic medication, the alterations in striatal dopaminergic neurotransmission of monogenic forms of PD are indistinguishable from those of common sporadic PD. This shared neurochemical phenotype underscores the pathophysiological overlap between idiopathic PD and monogenic forms of parkinsonism.

Radiotracer imaging of non-manifesting mutation carriers

This next section deals with the preclinical stages, as can be found in non-manifesting mutation carriers. Only a few PET or SPECT studies have examined such non-manifesting mutation carriers. Subtle but consistent pre-synaptic abnormalities in striatal dopaminergic neurotransmission were demonstrated in non-manifesting carriers of single heterozygous mutations in the parkin gene (PARK2) and PINK1 gene (PARK6), as indexed by a mild decrease in striatal DAT binding (Pellecchia et al., 2007) or pre-synaptic [18F]FDOPA uptake (Hilker et al., 2001, Khan et al., 2002a, Khan et al., 2002b). In LRRK2 mutations (PARK8), PET imaging with multiple tracers showed reduced striatal DAT binding in two non-manifesting mutation carriers, while another two were initially normal but later developed a decrease in DAT binding after 4 years of follow-up (Adams et al., 2005). Notably, the reduction in striatal DAT binding occurred in the absence of any change in striatal [11C] Raclopride binding in dopaminergic drug-naïve patients with a parkin mutation (PARK2), who showed a uniform decrease in striatal [11C] Raclopride binding indicating a symmetric postsynaptic alteration in striatal neurotransmission (Hilker et al., 2001). This finding was confirmed in a different group of patients who

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n=3) and LRRK2 gene (PARK8, n=2) (Samii et al., 1999, Adams et al., 2005, Kessler et al., 2005). A notable exception were levodopa treated patients with a Parkin mutation (PARK2), who showed a uniform decrease in striatal [11C] Raclopride binding indicating a symmetric postsynaptic alteration in striatal neurotransmission (Hilker et al., 2001). This finding was confirmed in a different group of patients who
An alteration in pre-synaptic dopaminergic function has not always been found in non-manifesting mutation carriers: a non-manifesting homozygous carrier of a mutation in the DJ1 gene (PARK7) showed reduced striatal DAT binding, but not her non-manifesting heterozygous brother (Hering et al., 2004). Moreover, three non-manifesting carriers of a mutation in the SNCA gene (PARK1) showed normal striatal DAT binding (Ahn et al., 2008).

Conversely, postsynaptic dopaminergic function seems to be normal in non-manifesting carriers. No changes in postsynaptic D2 receptor binding were found in eight non-manifesting mutation carriers in the SNCA gene (PARK1, n=1, (Kruger et al., 2001); Parkin gene (PARK2, n=5, (Hiiker et al., 2001)) and PINK1 gene (PARK6, n=3, (Kessler et al., 2005)).

In summary, despite the observed inter-individual variability, the crucial implication here is that the presence of a mutation in one of the PD genes may lead – in at least some of the non-manifesting mutation carriers – to a deficit in presynaptic dopaminergic transmission, but without leading to the hypokinetic-rigid features characteristic of PD. This latent presynaptic dysfunction, which is presumably caused by a subclinical neurodegeneration of nigrostriatal dopaminergic afferents, could be a threshold issue – not enough dopamine was lost in these subjects to render them symptomatic – but could also point to the presence of adaptive plasticity, either in the dopaminergic system or elsewhere. The evidence so far point towards activation of adaptive presynaptic – but apparently not postsynaptic – mechanisms to compensate for the loss of dopamine in the striatum.

Complementary neuroimaging techniques such as functional MRI can further tackle such issues, as we will address later.

**Structural magnetic resonance imaging**

With the advent of voxel based morphometry (VBM), morphometric analysis of high-resolution MRI scans has emerged as an objective tool to screen for subtle changes in local brain structure in a wide range of normal and pathological conditions (Ashburner and Friston, 2000, Good et al., 2001). Non-manifesting heterozygous carriers of a Parkin or PINK1 mutation display a bilateral increase in grey matter volume in the posterior putamen and internal globus pallidus (Binkofski et al., 2007). This result was also obtained when performing a standard region-of-interest analysis. Since most of the Parkin mutation carriers had previously been examined with $^{[18F]}$FDOPA PET, it was possible to test whether regional changes in presynaptic dopaminergic function correlated with regional changes in grey matter as revealed by structural MRI. It turned out that regional increases in striatal grey matter volume showed an inverse linear relationship with regional decreases in $^{[18F]}$FDOPA uptake (Binkofski et al., 2007). It was proposed that this “morphometric fingerprint” in the dysfunctional striatum may reflect an adaptive mechanism that helps to compensate for the latent nigrostriatal dysfunction. The morphometric finding also has an important implication as the regional increase in grey matter volume needs to be taken into account when interpreting changes in striatal dopaminergic function as revealed by $^{[18F]}$FDOPA PET.

These findings raise an important further issue, namely whether the “compensatory” increase in striatal grey matter volume persists if a mutation becomes clinically symptomatic. A cross-sectional VBM study which included symptomatic carriers of a Parkin mutation and patients with idiopathic PD suggest that this “hypertrophy” can be maintained only partially in the symptomatic stage of PD. Regression analyses which tested for linear changes in striatal grey matter as a function of severity and duration of the disease revealed bilateral grey matter decreases in the basal ganglia in symptomatic Parkin mutation carriers, suggesting that the basal ganglia are subject to a progressive atrophy, which gradually increases with disease severity and duration. However, this question needs to be addressed prospectively by performing repeated structural MRI images in asymptomatic mutation carriers as they become symptomatic (Reetz et al., 2008a).

Another important application of structural MRI is to link specific features of the clinical phenotype to regional changes in brain structure. In a recent study, a morphometric MRI study searched for structural correlates of psychiatric symptoms in 14 manifesting carriers of a PINK1 mutation and 14 healthy controls without mutation (Reetz et al., 2008b). Mutations in the PINK1 gene can cause parkinsonism and are frequently associated with psychiatric symptoms that may even precede motor symptoms and signs (Hedrich et al., 2006, Steinlechner et al., 2007). This group of PINK1 mutation carriers presented with a variety of psychiatric disorders, including major depression without psychotic symptoms (N=5), schizophrenia-spectrum disorders (N=4), panic disorder (N=2), adjustment disorder (N=2) and obsessive-compulsive personality disorder (N=2). Statistical comparison between all PINK1 mutation carriers and controls demonstrated a reduction in grey matter in the hippocampus and parahippocampus. Multiple regression analysis considering all psychiatric subscores simultaneously displayed degeneration patterns of different frontal and limbic structures.

**Mapping the consequences of mutation in PD genes on brain function**

Another line of recent research addressed how a mutation in one of the PD genes might affect functional brain networks. A wide array of complementary brain mapping techniques are available for this purpose, including functional MRI (fMRI), event-related cortical potentials (ERPs), or transcranial magnetic stimulation (TMS). Functional MRI (fMRI) is probably the most commonly used imaging modality, and maps regional changes in the blood oxygen level dependent (BOLD) signal to
assess the spatiotemporal pattern of brain activation during an experimental task (Logothetis, 2008). In patients with idiopathic PD, fMRI and ERP recordings have been used extensively to examine how the neurodegenerative process affects the function in motor, cognitive and limbic territories of the cortico-basal ganglia thalamocortical loops and how these abnormalities can be normalized by therapeutic interventions (van Eimeren and Siebner, 2006). With respect to monogenic forms of PD, the question arises whether the genetic forms of PD are associated with similar or different patterns of functional reorganization as sporadic PD. It is also interesting to know whether the “functional phenotypes” as revealed by functional brain mapping differ in monogenetic forms of PD depending on the affected gene. Another open question is whether genetic forms of PD are associated with unique response profiles of functional brain networks to acute or long-term dopamine replacement therapy and other therapeutic interventions. These functional imaging studies are currently underway, but no results have been published yet.

Several PET studies have provided converging evidence for a latent presynaptic deficit of nigrostriatal dopaminergic neurotransmission in non-manifesting mutation carriers of mutations in the Parkin, PINK1 and LRRK2 gene (Table 3). Therefore, functional neuroimaging of non-manifesting mutation carriers opens up the unique opportunity to examine how the cortico-basal ganglia-thalamocortical loops cope with a latent nigrostriatal dysfunction. One intriguing question is whether adaptive functional changes remain confined to the basal ganglia or also involve the cortex. Another interesting question is whether functional changes in cortico-basal ganglia-thalamocortical loops follow a stereotypical pattern across different experimental tasks or whether these changes differ depending on the cognitive processes that are required to perform a given experimental task.

In a first study, fMRI was employed to assess movement-related neuronal activity while 12 non-manifesting heterozygous carriers of a Parkin mutation and 12 healthy non-carriers performed visually paced thumb-to-finger opposition movements (Buhmann et al., 2005). In different blocks, participants had to select each movement themselves (i.e., internally cued movements) or movements were determined by the visual cue (i.e., externally cued movements). This task was chosen because patients with sporadic PD show under activity of the rostral supplementary motor area (SMA) and right dorsolateral prefrontal cortex during the internal selection but not externally determined finger movements (Playford et al., 1992). The presence of a Parkin mutation did not affect task performance, but task related activation was significantly influenced by the genotype. Non-manifesting mutation carriers showed a stronger increase in movement-related activity with internally selected movements in the right rostral cingulate motor area and left dorsal premotor cortex. The rostral cingulate motor area also showed stronger coupling with the left posterior putamen in Parkin mutation carriers relative to controls. Critically, these changes in task-related activity and coupling were only present if movements relied on internal cues. From these results it was inferred that in non-manifesting Parkin mutation carriers, the latent nigrostriatal dysfunction triggered a compensatory reorganisation of striatocortical loops to maintain normal performance (Buhmann et al., 2005).

Following-up on the study by Buhmann et al (2005), a recent fMRI study examined how a heterozygous mutation in a gene linked to recessively inherited Parkinson’s disease alters functional brain networks sub serving simple sequential movements. The study included non-manifesting individuals carrying a single mutant allele in the Parkin (n = 13) or PINK1 (n = 9) gene and two healthy control groups without mutation (van Nuenen et al., 2008). During fMRI, participants had to quickly perform a brief “chunk” of three movements, which were all determined by external cues. In Parkin or PINK1 mutation carriers, the simple motor sequence task consistently activated the rostral supplementary motor area (pre-SMA) and right rostral dorsal premotor cortex (PMd) which are specialized for the control of complex motor sequences. These premotor areas were not activated in control subjects without mutation. The additional recruitment of pre-SMA and rostral PMd was independent of the underlying genotype and occurred in the absence of any change in task performance. Taken together, the fMRI studies by Buhmann et al. (2005) and van Nuenen et al. (2008) consistently show that a preclinical dopaminergic deficit in the striatum alters corticobasal processing during motor tasks that rely on an intact function of the basal ganglia. The extra-recruitment of premotor areas seems to constitute a “generic” compensatory mechanism to maintain normal motor performance in the context of a latent nigrostriatal dopaminergic dysfunction. While these studies show that functional brain mapping provides a valuable tool of tracing adaptive changes in the motor system in non-manifesting mutation carriers, several issues remain to be addressed. It is likely that the latent dopaminergic dysfunction also results in compensatory changes in cortico-basal ganglia-thalamocortical loops that subserve more cognitive or limbic brain functions. Moreover, the latent dopaminergic deficit should also alter neuronal processing in the basal ganglia. A crucial question is how these adaptive changes dynamically develop during lifetime and which factors trigger a break-down of effective compensation. Future fMRI studies need to select appropriate experimental tasks and employ repeated fMRI measurements to tackle these issues.

Normal genetic variations in the general population

Given the multifactorial nature of PD, a likely scenario is that several genetic variants in the same or in different genes act together to confer susceptibility, although each variant alone is not pathogenic (Singh et al., 2008). In addition, many genetic
Table 3  Molecular imaging in monogenetic forms of parkinsonism.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Imaging technique</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sporadic Parkinson Disease</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAT</td>
<td>SPECT: (99mTc)-TRODAT-1, PET: [18F]FDOPA</td>
<td>↓ striatal DAT binding potential</td>
</tr>
<tr>
<td>Pre-synaptic</td>
<td>PET: [18F]FDOPA</td>
<td>↓ [18F]FDOPA uptake, putamen &gt; caudatus, contralateral to affected side</td>
</tr>
<tr>
<td>Post-synaptic</td>
<td>PET: [18F]FDOPA</td>
<td>Mild ↑ in D2 site availability in putamen</td>
</tr>
</tbody>
</table>

**Symptomatic mutation carriers**

| DAT | PET: [18F]FDOPA |↓ striatal uptake on both sides, putamen > caudatus (n=2) |

| PARK1 | PET: [18F]FDOPA |↓ striatal uptake on both sides, putamen > caudatus, more contralateral to affected side |
| PARK2 | SPECT: [11C]IBZM | ↓ striatal uptake (n=6) |
| PARK6 | PET: [18F]FDOPA |↓ striatal uptake on both sides, putamen > caudatus, symmetric (n=3) |
| PARK7 | PET: [18F]FDOPA |↓ striatal uptake on both sides, putamen > caudatus, more on contralateral side (n=1) |
| PARK8 | PET: [18F]FDOPA |↓ striatal uptake on both sides, putamen > caudatus (n=6) |

| Pre-synaptic | PET: [18F]FDOPA |↓ [18F]FDOPA uptake in putamen > caudatus |
| PARK1 | PET: [18F]FDOPA |↓ [18F]FDOPA uptake in putamen > caudatus, symmetric ↓ and slower ↓ of uptake compared to sPD |
| PARK2 | PET: [18F]FDOPA |↓ [18F]FDOPA uptake in putamen > caudatus, more symmetric ↓ and slower ↓ of uptake compared to sPD |
| PARK6 | PET: [18F]FDOPA |↓ [18F]FDOPA uptake in putamen > caudatus, posterior putamen = sPD anterior putamen & caudatus > sPD |
| PARK7 | PET: [18F]FDOPA |↓ [18F]FDOPA uptake in putamen > caudatus, more on contralateral side, slower ↓ of uptake than in sPD |
| PARK8 | PET: [18F]FDOPA |↓ [18F]FDOPA uptake in putamen > caudatus (n=4) |

| Post-synaptic | PET: [18F]FDOPA |↓ striatal uptake on both sides, putamen > caudatus (n=2) |
| PARK8 | PET: [18F]FDOPA |↓ striatal uptake on both sides, putamen > caudatus (n=10) |
| PARK9 | PET: [18F]FDOPA |↓ striatal uptake on both sides, putamen > caudatus (n=4) |

| Asymptomatic mutation carriers | PET: [18F]FDOPA |↓ striatal uptake (n=5) |
| PARK2 | PET: [18F]FDOPA |↓ striatal uptake (n=6) |
| PARK6 | PET: [18F]FDOPA |↓ striatal uptake (n=3) |

variants may modify the penetrance or onset of PD or may only influence specific aspects of PD such as the response to medication or contributing to the phenotypical heterogeneity (e.g. expression of specific symptoms). This renders it difficult to identify genetic susceptibility factors, quantify their effect size, and disentangle the complex interactions among them (Klein and Lohmann-Heddrich, 2007).

In healthy individuals, a vast body of neuroimaging data has been accumulated that normal variations in a single gene can explain inter-individual differences in regional brain activation when subjects perform experimental tasks (Goldberg and Weinberger, 2004, Mattay and Goldberg, 2004). For example, several single nucleotide polymorphisms (SNPs) in genes that regulate monoaminergic neurotransmission have been linked to distinct cognitive profiles, personality traits, and functional phenotypes as revealed by brain mapping (Volavka et al., 2004, Savitz et al., 2006).

Therefore, it can be assumed that common genetic variations may also influence cognitive functions, personality and brain network activity in patients with PD. It is likely though that these influences will differ from those found in the normal population because the neurodegenerative process may alter the susceptibility of functional brain networks to the functional consequences of a given polymorphism. Especially common polymorphisms that impact on dopaminergic signalling may exert disease-specific effects in patients with PD, for instance by influencing the functional brain response to dopaminergic therapy. In addition, the functional effects of common polymorphisms are likely to differ in motor, cognitive, and limbic cortico-striato-thalamo-cortical circuits given the rostro-caudal gradient of nigrostriatal neurodegeneration in PD.

Functional neuroimaging can be used to characterize gene-disease interactions between, on the one hand, PD and, on the other hand, common genetic polymorphisms that influence dopaminergic neurotransmission (Bartres-Faz et al., 2007, Williams-Gray et al., 2007, Williams-Gray et al., 2008). Two recent fMRI studies focused on a common functional polymorphism (val(158)met) within the catechol O-methyltransferase (COMT) gene which mainly impacts on prefrontal dopamine signalling (Weinberger et al., 2001). In a first fMRI experiment, patients with early sporadic PD who were homozygous for either valine (val) or methionine (met) were studied while they performed an executive task comprising both Tower of London (planning) and simple subtracting (“control”) problems during fMRI (Bartres-Faz et al., 2007). Val/val homozygotic patients with high COMT activity used a typical strategy, displaying preferential shifts in attention within rather than between dimensions. In contrast, met/met homozygotes with low COMT activity failed to form such an “attentional set” along with a reduced activation of the frontoparietal attentional network. The functional effects of COMT genotype on attentional strategy and brain activation interacted with dopaminergic medication (Williams-Gray et al., 2008). A second fMRI experiment used a task which required shifts in attention to demonstrate an interactive effect between the COMT val(158)met polymorphism and dopaminergic medication (Bartres-Faz et al., 2007, Williams-Gray et al., 2007, Williams-Gray et al., 2008). This study included 29 medicated patients with early PD. Val/val homozygotic patients with high COMT activity used a typical strategy, whereas met/met homozygotes with a low COMT activity displayed preferential shifts in attention within rather than between dimensions. This suggests that the COMT genotype modulates executive function in PD through a direct influence on frontoparietal activation (Williams-Gray et al., 2007).

Conclusion

The combined neurogenetics-neuroimaging approach has already started to yield results that shed new light on the pathophysiology of PD. Maybe the best example is the multimodal assessment of brain structure and function in non-manifesting carriers with a single mutant Parkin allele (Fig.1). Although the results need to be confirmed in larger cohorts of mutation carriers, the different imaging modalities provided important pieces to resolve the puzzle of preclinical reorganization of PD. Together these studies added weight to the hypothesis that the presence of a single mutant Parkin allele does affect nigrostriatal dopaminergic function which triggers adaptive changes in motor cortical areas (Fig.1). Similar multimodal studies are warranted in other monogenic forms of PD. One pertinent question is which neurobiological factors reduce the penetrance of mutations in the LRRK2 gene. Another promising line of research is to evaluate the functional consequences of common genetic polymorphisms on brain function or response to treatment. The feasibility of this approach has been demonstrated for the (val(158)met) polymorphism
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in the COMT gene, and motivates further studies on the impact of other common genetic polymorphisms that modify monoaminergic neurotransmission or influence neuronal plasticity. It remains a challenge, however, how to deal with multiple polymorphisms and their complex interactions with environmental factors (e.g. exposure to neurotoxins) and normal aging.

While previous studies have been cross-sectional, prospective longitudinal assessment of genotype-phenotype interactions are warranted to identify the role of genetic factors in various stages of PD. Neuroimaging will also play a role in the therapeutic application of genetics in PD. For instance, gene transfer therapy is currently considered as a new therapeutic option in PD (Kordower et al., 2006, Emborg et al., 2007, Feigin and Eidelberg, 2007, Kaplitt et al., 2007, Eberling et al., 2008, Marks et al., 2008). Here neuroimaging techniques may be valuable to assess the consequences of these new interventional therapies on brain function (Feigin et al., 2007).

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Abbreviations

(p)PD (Sporadic) Parkinson Disease
SN Substantia Nigra
6-OHDA 6-Hydroxy-dopamine
MPTP 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
SNCA α-Synuclein
LRRK2 Leucine Rich Repeat Kinase 2
PINK1 PTEN-induced putative kinase 1
OMIM Online Mendelian Inheritance in Man
PET Position Emission Tomography
SPECT Single Photon Emission Computed Tomography
[Tc]FDOPA 6-[(+)-fluoro-L-3,4-dihydroxyphenylalanine
[Tc]FP-CIT 123I-Fluorpropyl-Carbamothexy-3-[(4-iodophenyl)-Tropane
[Tc]TRODAT-1 123I-2-carbomethoxy-3-(4-iodophenyl)-Tropane
DAT Depamine Transporter
IBZM 3%-iodobenzamide
[123I]QMP 123I-o-3-thrio-methylphenylate
GP1 Globus Pallidus Internus
Put Putamen
MRI Magnetic Resonance Imaging
fMRI Functional Magnetic Resonance Imaging
VBM Voxel-Based Morphometry
BOLD Blood Oxygen Level Dependent
SMR Supplementary Motor Area
PMd Dorsal Premotor Cortex
tCMA Rostral Cingulate Motor Area
TCS Transcranial Sonography
GSP1 Glutathione S-Transferase pi 1
DRD2 D2 Receptor Gene
COMT Catechol-O-Methyltransferase
ID Intradimensional
ED Extradimensional
CNS Central Nervous System
Val Valine
Met Methionine
AAS Adenos-Associated Virus
PEG Polyethyleneglycol
PIL Polyethyleneglycolylated Immuno Liposome
hAAADC (human) Aromatic L-Amino Acid Decarboxylase
ERPs Event-Related Cortical Potentials
TMS Transcranial Magnetic Stimulation
SNP Single Nucleotide Polymorphism

References


Mapping preclinical compensation in Parkinson’s disease: an imaging genomics approach

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Abstract

Mutations in the Parkin (PARK2) and PINK1 gene (PARK 6) can cause recessively inherited Parkinson’s disease (PD). The presence of a single Parkin or PINK1 mutation is associated with a dopaminergic nigrostriatal dysfunction and conveys an increased risk to develop PD throughout lifetime. Therefore neuroimaging of non-manifesting individuals with a mutant Parkin or PINK1 allele opens up a window for the investigation of preclinical and very early phases of PD in vivo. Here we review how functional magnetic resonance imaging (fMRI) can be used to identify compensatory mechanisms that help to prevent development of overt disease. In two separate experiments, Parkin mutation carriers displayed stronger activation of rostral supplementary motor area (SMA) and right dorsal premotor cortex (PMd) during a simple motor sequence task and anterior cingulate motor area and left rostral PMd during internal movement selection as opposed to externally cued movements. The additional recruitment of the rostral SMA and right rostral PMd during the finger sequence task was also observed in a separate group of non-manifesting mutation carriers with a single heterozygous PINK1 mutation. Since mutation carriers were not impaired at performing the task, the additional recruitment of motor cortical areas indicates a compensatory mechanism that effectively counteracts the nigrostriatal dysfunction. These first results warrant further studies that use these imaging genomics approach to tap into preclinical compensation of PD. Extensions of this line of research involve fMRI paradigms probing non-motor brain functions. Additionally, the same fMRI paradigms should be applied to non-manifesting mutation carriers in genes linked to autosomal dominant PD. This will help to determine how “generically” the human brain compensates for a preclinical dopaminergic dysfunction.

Introduction

Several genes have been identified in which mutations can result in familial forms of Parkinson’s disease (PD) (Gasser, 2007, Klein and Lohmann-Hedrich, 2007, Klein et al., 2007). The genes and chromosomal loci linked to familial forms of PD have been designated as PARK1–13. These loci include six autosomal dominant (PARK1(=4), 3, 5, 8, 11 and 13), four recessive (PARK2, 6, 7, and 9), one X-linked (PARK12) and one form with an as yet unknown mode of transmission (PARK10). Although monogenic forms account for less than 5% of all patients presenting with PD, they have considerably stimulated research on PD. The identification of the gene products of the PARK genes and research into the relevance of these proteins to cellular function have considerably advanced our understanding of the molecular mechanisms causing PD (Gasser, 2007).

Recent research has also started to examine how the alterations at the cellular level induced by a mutation in a PARK gene impact on human brain function in vivo. This question can be addressed using functional magnetic resonance imaging (fMRI). Blood oxygen level-dependent (BOLD) fMRI is probably the most widely used functional neuroimaging technique. Regional changes in BOLD signal assay the spatiotemporal pattern of brain activation during an experimental task (Logothetis, 2008). As such, fMRI offers a sensitive method of mapping the functional impact of inter-individual genetic variations on neuronal processing within distinct brain regions and circuits (Goldberg and Weinberger, 2004). In individuals who carry a PARK mutation, fMRI can be used to identify how a given mutation changes the spatiotemporal patterns of brain activity subserving a specific type of behaviour (van Eimeren and Siebner, 2006). The potential of fMRI goes beyond studies that solely rely on behavioural tests or clinical examination. The fMRI approach can be used to examine where in the brain, under which condition, and how a given PARK mutation alters neuronal processing in functional brain networks. Since genotype-specific changes in regional neuronal activity can be demonstrated without any observable change in behaviour, the neuroimaging-based approach is also more sensitive to identify genotype-specific traits in the human brain (Hariri and Weinberger, 2003). In this review, we summarize our recent fMRI results obtained in non-manifesting heterozygous carriers of a Parkin (PARK2) or PINK1 (PARK6) mutation.

Single heterozygous mutation in the Parkin and PINK1 gene

Mutations of the Parkin gene (PARK2) and PINK1 gene (PARK6) are among the most common monogenic causes of early-onset PD (Gasser, 2007). Although there might be some characteristic clinical features as a group, it is impossible to distinguish individual PD patients carrying homozygous or compound heterozygous mutations in the Parkin or PINK1 gene from individual sporadic PD cases without
mutations (Albanese et al., 2005, Bonifati et al., 2005, Hedrich et al., 2006). For a detailed review of Parkin or PINK1 associated PD, we refer to two recent reviews (Gasser, 2007, Klein et al., 2007).

Given the recessive mode of inheritance of Parkin and PINK1 associated PD, one might expect that family members carrying only a single mutant allele should be spared. Recent research, however, suggests that a single mutant allele may also predispose to PD, although with a later onset and a much lower penetrance (Klein et al., 2007). Moreover, adult non-manifesting carriers of a single mutant Parkin or PINK1 allele showed a mild reduction of presynaptic 18F-Dopa uptake in the striatum (Hilker et al., 2001, Khan et al., 2005). The reduced striatal 18F-Dopa uptake indicates a subclinical nigrostriatal dopaminergic degeneration and confirms that a single mutant allele increases the susceptibility to late-onset PD. A single heterozygous mutation in the Parkin or PINK1 gene also alters the structure of the basal ganglia: Adult non-manifesting mutation carriers show a relative increase in grey matter volume of the posterior putamen and globus pallidus (Binkofski et al., 2007). In addition, transcranial ultrasound revealed hyperechogenicity of the substantia nigra (SN) in non-manifesting heterozygous Parkin mutation carriers, just as in patients with sporadic PD where SN hyperechogenicity is present in 90% of cases (Walter et al., 2004). In sporadic PD, it is assumed that the neurodegenerative process starts at least several years before affected individuals become symptomatic, although estimates of the preclinical period remain difficult (Braak et al., 2003). Importantly, the presence of such a preclinical period suggests that the human motor system has a substantial potential to compensate for the slowly progressive nigrostriatal dopaminergic denervation (Forno, 1969, Fearnley and Lees, 1991, Koller, 1992). However, the mechanisms that mediate functional compensation and delay the onset of overt PD are difficult to study in sporadic cases because a reliable method to identify individuals at risk for developing sporadic PD is not yet available. In this context, non-manifesting individuals carrying a single heterozygous mutation in the Parkin or PINK1 gene represent a rare but unique opportunity to study the preclinical pathophysiology of PD, because the mutation conveys an increased risk for PD and is associated with a latent nigrostriatal dysfunction. An additional advantage is that presymptomatic mutation carriers are not impaired at performing specific motor tasks, allowing for assessment of task-related cerebral activations without the confounding influence of differences in behavioural performance (a factor that complicates the interpretation of such studies in patients with overt disease).

Methodological considerations

In the following sections, we summarize the results of two recent fMRI studies that were conducted to tap into disease-specific mechanisms that might compensate for subclinical nigrostriatal degeneration. Our central hypothesis was that non-manifesting carriers of a single mutant allele should reveal changes in task-related activation during motor tasks that reflect the latent nigrostriatal dysfunction and preclinical compensation. Before describing this in more detail, we would like to summarize some general considerations which guided the design of our fMRI experiments.

Motor symptoms represent the clinical hallmark of sporadic PD. This is because nigrostriatal dopaminergic degeneration is most prominent in the motor loops of the cortico-basal ganglia thalamo-cortical circuits, especially in the early stage of PD (Gibb, 1997, Blaindini et al., 2000). Therefore, we decided to use experimental tasks in our fMRI studies that primarily probed specific aspects of motor control. When choosing our experimental motor tasks, three selection criteria were applied. First, we chose motor tasks that probed different aspects of manual motor control. This enabled us to test whether the presence of a Parkin or PINK1 mutation triggers functional changes in distinct motor brain networks that depend on the context of manual motor control. Second, we opted for motor tasks which have been extensively studied in patients with overt PD since we wished to interpret our fMRI results as closely as possible in the pathophysiological context of PD. Third, we deliberately selected simple motor tasks that could be easily performed by each participant. This ensured that task performance was matched among groups with different genotypes excluding any confounding effect of altered task performance. It also maximized our chances to identify motor regions that were additionally recruited in mutation carriers by avoiding any ceiling effect in terms of task-related activation.

fMRI of internal movement selection

Our first fMRI study included 12 non-manifesting carriers of a single heterozygous Parkin mutation and 12 healthy age-matched non-carriers (Buhmann et al., 2005). The experimental task required the selection of single thumb-to-finger opposition movements which were performed with the right hand (Buhmann et al., 2005). The onset of each movement was visually paced at a low rate. The task consisted of separate blocks in which participants had to select each movement themselves (internally cued) or execute a movement as specified by the visual cue (externally cued; figure 1A).

This type of task probes the function of motor areas involved in movement selection (Samuel et al., 1997, Sabatini et al., 2000), and has been applied to patients with symptomatic PD (Playford et al., 1992, Georgiou et al., 1994, Jahanshahi et al., 1995). In PD patients, behavioral and neuroimaging studies have provided consistent evidence that internally selected movements are particularly affected by the basal ganglia dysfunction and results in an underactivity of the rostral supplementary
mutation carriers, the right rostral cingulate motor area (rCMA) and left dorsal premotor cortex (PMd) showed a stronger increase in movement-related activity when movements had to be internally selected relative to externally instructed movements. The rCMA also showed stronger coupling with the dopamine-deficient left posterior putamen in Parkin mutation carriers. Again, these changes in task-related coupling were only present if movements relied on internal cues. These alterations in task-related activation suggest that in non-manifesting Parkin mutation carriers, the latent nigrostriatal dysfunction triggers a compensatory recruitment of cortical motor areas along with a change in inter-regional connectivity to maintain normal performance (Buhmann et al., 2005).

motor area (SMA) and right dorsolateral prefrontal cortex with internal movement selection (Playford et al., 1992, Georgiou et al., 1994, Jahanshahi et al., 1995). While there were no differences in task performance between non-manifesting carriers of a Parkin mutation and controls without Parkin mutation, fMRI showed a significant influence of the genotype on task-related motor activity. In non-manifesting
**fMRI of sequential finger movements**

While our first study identified changes in neuronal activity depending on the mode of movement selection in non-manifesting carriers of a single mutant Parkin allele (Buhmann et al., 2005), two important questions were not addressed. Since only carriers with a Parkin mutation were studied, it remained unclear whether the enhanced recruitment of frontal motor areas constitutes a compensatory mechanism specific to mutations in the Parkin gene or reflects a “generic” response pattern that can be triggered by any mutation affecting nigrostriatal dopaminergic function. Another open question was whether motor compensation always recruits the same premotor regions across a range of different motor tasks. Alternatively, functional compensation might be more flexible recruiting different sets of premotor areas depending on the specific task demands.

To address these issues, the second fMRI study (van Nuenen et al., 2009b) included non-manifesting carriers of a mutation in either the PINK1 or Parkin gene. Given the closely related dysfunctional effects of both proteins (Clark et al., 2006, Park et al., 2006, Yang et al., 2006, Exner et al., 2007, Kim et al., 2008), our prediction was that the functional phenotype at a brain network level would be identical for both groups. We also modified the motor task which now required participants to generate sequential rather than single finger movements (figure 1B). Movement sequences were simple and consisted of three thumb-to-finger opposition movements. Visual cues instructed participants which sequence to perform on each trial. We chose this simple motor sequence task for two reasons. First, sequential finger movements have been widely used to study motor control in idiopathic PD (Samuel et al., 1997, Catalan et al., 1999, Carbon and Eidelberg, 2006) showing compensatory overactivity in the PMd and intraparietal sulcus. Second, neuroimaging studies in healthy individuals have identified frontal motor areas which subserve complex as opposed to simple movements. In healthy subjects, the rostral part of the supplementary motor area (referred to as pre-SMA) and the rostrodorsal portion of the right PMd (Sadato et al., 1997, Boecker et al., 1998) show a linear increase in regional activity with increasing complexity of the motor sequence. Therefore we were able to make specific predictions regarding the regional pattern of compensatory activation. We expected that the latent dopaminergic dysfunction in non-manifesting carriers of a Parkin or PINK1 mutation will call on a compensatory overactivity in the pre-SMA and right PMd during the simple sequential finger movement task.

As in our first fMRI study on movement selection (Buhmann et al., 2005), mutation carriers and controls without mutation performed the task equally well. Non-manifesting Parkin or PINK1 mutation carriers consistently activated the pre-SMA and right rostral PMd when they made simple sequential finger movements. These areas were not activated in the control groups without mutation. Taken together, the two fMRI experiments indicate that the otherwise preclinical nigrostriatal dopaminergic denervation that is present in individuals with a Parkin or PINK1 mutation is indeed functionally relevant. It appears to affect those aspects of motor control that rely on intact basal ganglia function such as internal movement selection or sequencing of movements. At least for relatively simple motor tasks, this dysfunction remains subclinical because additional motor areas are recruited during task performance. In other words, the motor system works harder to effectively compensate for the basal ganglia dysfunction. Compensatory recruitment involves motor cortical areas within the pre-existing network, and thus, the spatial pattern of increased cortical activity critically depends on the motor networks that subserve the experimental task. For instance, performing simple sequential movements led to increased activation in areas which are critical to the control of complex motor sequences in healthy subjects (Sadato et al., 1997, Boecker et al., 1998).

We used the psycho-physiological interactions (PPI) method described by Friston et al (Friston et al., 1997) to further characterize the compensatory role of rSMA and right PMd. Defining the rSMA and right PMd as region of interest, the PPI revealed no effect of the genotype on task-dependent changes in functional coupling between the overactive regions and other motor brain regions. The implication is that the overactive premotor regions were able to draw on a normal pattern of connectivity. A second more speculative interpretation would be that no increase in functional coupling was needed to ensure a normal level of performance. An increase in coupling might be engaged if the task would require the performance of more complex motor sequences. Overactivity of pre-SMA and rostral PMd was equally present in PINK1 and Parkin mutation carriers. Since the compensatory recruitment of pre-SMA and rostral PMd was independent of the underlying genotype, we argue that the observed activation constitutes a “generic” compensatory mechanism to maintain normal sequencing of movements in the context of a mild dopaminergic deficit. However, PINK1 mutation carriers showed an additional mainly left-hemispheric recruitment of frontoparietal areas that was not present in Parkin mutation carriers. It is unclear whether this reflects a true genotype-specific pattern of functional adaptation in the frontoparietal cortex. Alternatively, non-manifesting PINK1 mutation carriers might have a stronger functional impairment of the motor system, calling on additional frontoparietal loops during task performance. Another caveat is that all these results were obtained from members of two families, so there may be observations which are family-specific. Whatever the cause of the difference in the compensatory recruitment of cortical motor areas, it underscores the capacity of human sensorimotor networks to flexibly adapt to a nigrostriatal dysfunction.
Impact of task performance

In the fMRI experiment on sequential finger movements, participants were instructed to perform the sequential finger movement as fast as possible, but also as accurately as possible. This resulted in a considerable inter-individual variation of mean movement time, presumably because participants adopted different strategies when performing the task. To examine whether genotype-driven changes in motor activity in the presence of a Parkin or PINK1 interacted with the speed of task performance, we divided both mutation carriers and non-mutation carriers into fast and slow performers. Median movement time was approximately 1500 ms. Therefore, all participants with a mean movement time more than 1500 ms were defined as “slow performers”, while participants who had a mean movement time less than 1500 ms were considered “fast performers”. This resulted in four groups: fast mutation carriers (fast Mut+), slow mutation carriers (slow Mut+), fast non-mutation carriers (fast Mut‐), slow non-mutation carriers (slow Mut‐). Overactivity in the rSMA and right PMd was independent of the speed of performance (Figure 2). Fast mutation carriers showed increased activation of the left primary motor hand area (M1-Hand) relative to fast non-mutation carriers, whereas slow mutation carriers showed stronger activity of the left putamen compared with slow non-mutation carriers (Fig.1). The activity profile showed an increased activity in all groups during the task except in fast non-mutation carriers in the left M1-Hand. In the left caudal putamen, slow mutation carriers showed more activity relative to the other three groups during the sequential task. These preliminary results indicate that the way participants perform the task does influence the effects of the genotype on movement-related activity. This issue needs to be taken into account in future studies on preclinical motor reorganization in non-manifesting carriers of a PARK mutation.

Dopaminergic therapy in Parkin-associated PD

We described compensational mechanisms in Parkin and PINK1 mutation carriers. But what happens when those mechanisms give way and formerly non-manifesting Parkin mutation carriers now become symptomatic? Patients with Parkin-associated PD often show a stable long-term response to dopaminergic therapy without developing motor fluctuations (Lohmann et al., 2003). Therefore, we reasoned that one may find differences in motor activation when comparing Parkin-associated and sporadic PD on dopaminergic medication. In an unpublished study, we tested this hypothesis using fMRI in nine medicated patients with Parkin-associated, eleven with sporadic PD, and ten healthy controls while they performed externally specified or internally selected finger movements. Both patient groups had similar disease duration, levodopa equivalent dose, and total motor scores of the United Parkinson’s Disease Rating Scale off dopaminergic medication (Parkin-associated PD: 20.9 ± 13.8; sporadic PD: 22.8 ± 12.8). All groups demonstrated that internal selection led to a stronger activation of a well described network of mesial and lateral frontal areas (pre-SMA, PMd and prefrontal cortex). Even with a very liberal
CHAPTER 3 MAPPING PRECLINICAL COMPENSATION IN PARKINSON’S DISEASE

This would allow estimating the functional relevance of these areas for the maintenance of a motor function. Due to its excellent temporal resolution, EEG and MEG can identify compensatory changes in the time course of task related motor activity. We anticipate that multimodal functional brain mapping will also be of great value in mapping preclinical reorganization of functional brain networks in individuals with a mutation in other PARK genes that lead to autosomal dominant PD (e.g. mutations in the LRRK2 gene). This would help to clarify whether or not the brain can “generically” compensate for preclinical dopaminergic dysfunction, in the context of a different genetic background. Another target for functional neuroimaging is to address how the latent nigrostriatal dysfunction in non-manifesting PARK mutation carriers triggers compensatory responses in cognitive and limbic brain networks. Finally, longitudinal fMRI studies need to clarify whether the compensatory mechanisms as identified with fMRI persist, increase or attenuate in mutation carriers who ultimately develop PD. A recent PET activation study of patients with sporadic PD suggests that compensatory extra-recruitment of frontal motor areas persists at least in the early stage of the disease (Mentis et al., 2003).

Methodological considerations

While our results are encouraging, there are a few methodological issues that require some discussion. At a group level, asymptomatic PARK1 and Parkin mutation carriers display a latent dopaminergic depletion and have an increased risk of developing PD. However, there is currently no data on the natural course of potential clinical manifestations resulting from a heterozygous mutation in asymptomatic individuals; in fact, only some of them may actually develop PD during their lifetime. Another shortcoming is that the carriers of the PARK1 (Family W) and Parkin (Family LA) mutation were all members of a large kindred. Therefore the results need to be replicated in a more heterogeneous sample of mutation carriers from different families. It would also be interesting to conduct a pharmacological fMRI study to examine whether the compensatory recruitment of motor brain regions can be readily reversed by a small dosage of levodopa. Another open question is how relevant the compensatory recruitment of the motor areas actually is to task performance.

Conclusions and outlook

Our recent fMRI studies in non-manifesting carriers of a single heterozygous mutation carriers of PARK1 or Parkin highlights the potential of a combined neurogenetic-neuroimaging approach to unravel the intrinsic potential of the human brain to compensate for a latent nigrostriatal dysfunction. Future extensions of this approach may employ other brain mapping techniques such as transcranial magnetic stimulation (TMS), electroencephalography (EEG) or magneto-encephalography (MEG). So far, TMS has only been used to assess excitability changes in sensorimotor circuits (Baumer et al., 2007). In the context of functional reorganization, TMS may also be used to transiently disrupt task-related processing in motor cortical areas that show compensatory activity. This would allow estimating the functional relevance of these areas for the maintenance of a motor function. Due to its excellent temporal resolution, EEG and MEG can identify compensatory changes in the time course of task related motor activity. We anticipate that multimodal functional brain mapping will also be of great value in mapping preclinical reorganization of functional brain networks in individuals with a mutation in other PARK genes that lead to autosomal dominant PD (e.g. mutations in the LRRK2 gene). This would help to clarify whether or not the brain can “generically” compensate for preclinical dopaminergic dysfunction, in the context of a different genetic background. Another target for functional neuroimaging is to address how the latent nigrostriatal dysfunction in non-manifesting PARK mutation carriers triggers compensatory responses in cognitive and limbic brain networks. Finally, longitudinal fMRI studies need to clarify whether the compensatory mechanisms as identified with fMRI persist, increase or attenuate in mutation carriers who ultimately develop PD. A recent PET activation study of patients with sporadic PD suggests that compensatory extra-recruitment of frontal motor areas persists at least in the early stage of the disease (Mentis et al., 2003).

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Heterozygous carriers of a Parkin or PINK1 mutation share a common functional endophenotype

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CHAPTER 4 COMPENSATION IN PARKIN- AND PINK1-PARKINSONISM

Abstract

Background: To use a combined neurogenetic-neuroimaging approach to examine the functional consequences of preclinical dopaminergic nigrostriatal dysfunction in the human motor system. Specifically, we examined how a single heterozygous mutation in different genes associated with recessively inherited Parkinson’s disease alters the cortical control of sequential finger movements.

Methods: Adult non-manifesting individuals carrying a single heterozygous Parkin (n=13) or PINK1 (n=9) mutation and 23 healthy controls without these mutations were studied with functional magnetic resonance imaging (fMRI). During fMRI, participants performed simple sequences of three thumb-to-finger opposition movements with their right dominant hand. Since heterozygous Parkin and PINK1 mutations cause a latent dopaminergic nigrostriatal dysfunction, we predicted a compensatory recruitment of those rostral premotor areas that are normally implicated in the control of complex motor sequences. We expected this overactivity to be independent of the underlying genotype.

Results: Task performance was comparable for all groups. The performance of a simple motor sequence task consistently activated the rostral supplementary motor area (pre-SMA) and right rostral dorsal premotor cortex (PMd) in mutation carriers but not in healthy controls. Task related activation of these premotor areas was similar in carriers of a Parkin or PINK1 mutation.

Conclusion: Mutations in different genes linked to recessively inherited Parkinson’s disease are associated with an additional recruitment of pre-SMA and rostral PMd during a simple motor sequence task. These premotor areas were recruited independently of the underlying genotype. The observed activation most likely reflects a “generic” compensatory mechanism to maintain motor function in the context of a mild dopaminergic deficit.

Introduction

Several genes have been identified that can lead to Parkinson’s disease (PD), including four recessively inherited forms caused by mutations in the Parkin (PARK2), DJ-1 (PARK7), PINK1 (PARK6), and the ATP13A2 (PARK9) gene (Kitada et al., 1998, Bonifati et al., 2003, Valente et al., 2004). These familial forms of PD show a substantial clinical overlap with sporadic PD. Non-manifesting individuals who carry a single heterozygous mutation in the Parkin and PINK1 gene associated with recessively inherited PD have attracted particular interest (Klein et al., 2007). Positron emission tomography (PET) of dopaminergic neurotransmission showed that these individuals have a mild presynaptic dopaminergic dysfunction in the striatum (Hilker et al., 2001, Khan et al., 2002a, Khan et al., 2002b, Khan et al., 2005). Therefore, non-manifesting carriers of a single mutant allele provide a unique model to study how a subclinical loss of dopamine-producing cells in the substantia nigra on the human motor system (van Eimeren and Siebner, 2006).

In a recent functional magnetic resonance imaging (fMRI) study we provided evidence for a compensatory redistribution of neuronal activity within the motor system in non-manifesting carriers of a heterozygous mutation in the Parkin gene. With internally cued movements, mutation carriers displayed a stronger activation of the right rostral cingulate motor area and left dorsal premotor cortex (PMd) compared to externally cued movements (Buhmann et al., 2005). They also showed stronger functional coupling between the rostral cingulate motor area and posterior putamen in the context of internal movement selection. Because mutation and non-mutation carriers performed the task equally well, these activity changes were interpreted as adaptive redistribution of neuronal activity in rostral motor cortical areas which helps to maintain motor function in the context of a latent nigrostriatal dysfunction (Buhmann et al., 2005).

The present experiment extended our previous fMRI study in two directions. First, we used a different experimental task which required participants to quickly perform a brief “chunk” of three movements. In our previous fMRI study, the experimental task required the selection of single movements. The onset of each movement was externally paced at a low rate and consecutive movements were separated by periods of rest. By using a “real” motor sequence task, we examined how a heterozygous mutation in a gene linked to recessively inherited Parkinson’s disease impacts on functional brain networks subserving sequential movements. We hypothesized that the regional expression of functional changes in motor cortical areas critically depends on the particular function probed by the experimental task. Therefore, the adaptive redistribution of cortical activity within pre-existing motor networks was expected to be different for the motor sequence task as opposed to the previously used movement selection task. Specifically, we predicted that
mutation carriers would show a compensatory recruitment of rostral premotor areas that are specialized for the control of complex motor sequences. Second, we included non-manifesting individuals carrying a single mutant allele in the Parkin or PINK1 gene. This enabled us to test whether the adaptive redistribution of neuronal activity in motor brain regions is specifically linked to mutations in a specific gene associated with recessively inherited Parkinson’s disease. Given the closely related dysfunctional effects of mutations in both proteins in a drosophila model (Clark et al., 2006, Park et al., 2006), our prediction was that the functional phenotype at a brain network level would be similar for both groups.

Materials and Methods
Participants. We studied thirteen subjects (mean age 38.9±5.8 years, 7 men) carrying a single heterozygous mutation in the Parkin gene, either a deletion of exon 7 (n=7) or a single base-pair deletion in exon 9 (c.del1072T) (n=6) (Pramstaller et al., 2002). Nine other subjects (mean age 41.9±5.7 years, 7 men) carried a heterozygous c.1366C>T nonsense mutation of the PINK1 gene (Hedrich et al., 2006) Three of the Parkin (Pramstaller et al., 2002, Pramstaller et al., 2005) and five of the PINK1 (Hedrich et al., 2006, Hiller et al., 2007) mutation carriers had minor motor signs upon careful clinical examination, but were not aware of the motor signs and motor signs did not interfere with their daily activities. None of these subjects had a UPDRS score of more than 4 or met the international accepted diagnostic criteria of probable PD. Nine of the heterozygous carriers of a Parkin mutation had previously undergone 18F-DOPA PET showing a presynaptic dopaminergic deficit in the striatum (Hilker et al., 2001).

We also studied two groups of healthy age-matched controls: 13 volunteers (mean age 38.7±5.5 years, 7 men) who served as controls for the non-manifesting Parkin mutation carriers and 10 volunteers (mean age 40.0 ± 5.9 years, 7 men) formed the control group for the non-manifesting PINK1 mutation carriers. Controls were recruited from a departmental register of volunteers and did not have mutations in Parkin or PINK1.

Participants had no history of a previous neuropsychiatric disease nor had they previously received dopaminergic or other anti-parkinsonian drug treatment. All participants were consistent right-handers according to the Edinburgh handedness inventory (Oldfield, 1971). Written informed consent was obtained prior to the study. The experimental procedures had the approval of the local ethics committee.

Experimental design. The fMRI experiment consisted of 10 alternating blocks of REST and TASK. During the TASK periods, participants repeatedly performed sequential finger movements with their right dominant hand. Each sequence consisted of three thumb-to-finger opposition movements instructed by external visual cues. Participants produced three different motor sequences in pseudorandom order. The details of the experimental task are given in Fig. 1a. Before fMRI, participants were familiarized with the task and practiced the respective finger sequences for approximately five minutes.

By choosing a short sequence, we kept the task simple favouring automatic performance without a high level of monitoring. The use of longer sequences would have increased the load on working memory, possibly forcing subjects to divide the sequence into separate “chunks” (Kennerley et al., 2004). We randomly presented three sequences rather than repeating the same sequence during a given block. This forced the participants to continuously switch between different motor representations of simple overlearned sequences.

Our decision to select sequential finger movements as experimental task was based on two considerations: First, sequential finger movements have been extensively studied in PD, providing evidence for compensatory overactivity in the PMd and intraparietal sulcus in PD during sequential movements (Samuel et al., 1997, Catalan et al., 1999, Carbon and Eidelberg, 2006). Second, healthy controls show a linear increase in activity with sequence complexity in the rostral part of the supplementary motor area (referred to as pre-SMA) and the rostrodorsal portion of the right PMd (Sadato et al., 1997, Boecker et al., 1998). Therefore, we hypothesized that the latent dopaminergic dysfunction in presymptomatic carriers of a Parkin or PINK1 mutation results in a compensatory recruitment of the pre-SMA and right PMd to maintain motor performance within a normal range.

Participants performed 30 consecutive sequences per fMRI session. To assess performance during fMRI, we taped aluminium foil to the tips of the thumb and the fingers of the right hand. When the thumb and finger tips contacted each other, an electrical circuit was closed which was specific to a given finger. For each trial, we recorded the time during which the tip of the thumb had contact with the index, middle, ring or little finger. This enabled us to calculate the time that elapsed between the first and last finger-to-thumb contact of the motor sequence, referred to as Tap1-Tap3 interval. To assess the stability of motor performance, we calculated the mean Tap1-Tap3 interval for ten consecutive trials during the fMRI session.

MRI data acquisition. Whole-brain MRI was performed on a 1.5 T Magnetom Symphony scanner (Siemens, Erlangen, Germany) equipped with a standard head coil. We used a T2*-weighted gradient-echo echoplanar sequence (TR = 3000 ms, TE = 40 ms, flip angle = 90°, matrix 64 x 64 voxels, field of view = 256 x 256 mm², 30 axial slices, slice thickness: 4 mm) to map task-related changes in the blood oxygen level dependent (BOLD) signal. 160 brain volumes were acquired per
The fMRI session consisted of ten alternating periods without movements (REST) or sequential movements (TASK). Each block lasted for 24 s. There were 10 blocks of TASK and 10 blocks of REST. A two-dimensional drawing of the palm of the right hand was continuously presented in the centre of the visual field throughout the fMRI session. During the REST periods, the line drawing of the hand was continuously presented but without dots. Participants were instructed to remain still and fixate the hand with their eyes. During each block of TASK, participants performed three motor sequences. Each sequence consisted of three thumb-to-finger opposition movements. At the onset of each movement trial, the index, middle, ring or little finger was labelled with a red dot on a two-dimensional drawing of the palm of the right hand. The position and order of the red dot specified the motor sequence that had to be performed within a given trial. When the instruction cue disappeared from the screen, participants sequentially tapped with the tip of their right thumb onto the tip of the indicated fingers. They were asked to move at a convenient speed and to perform the task as accurately as possible.

Data analysis. Using the mean Tap1-Tap3 interval as dependent variable, we performed a two-factorial repeated-measures analysis of variance (ANOVA) with

the within-subject factor TIME (three levels: trial 1 to 10, trial 11-20, and trial 21-30) and between-groups factor GROUP (4 levels: non-manifesting PINK1 or Parkin mutation carriers and their respective control groups without mutation). The Greenhouse-Geisser method was used to correct for non-sphericity if appropriate. Depending on a significant F-value, post-hoc t-tests were performed. Data are given as mean and onefold standard deviation. A p-value of <0.05 was considered significant.

The fMRI data were processed and analysed using statistical parametric mapping (SPM) software (SPM2; Wellcome Trust Centre for Neuroimaging, London, UK; http://www.fil.ion.ucl.ac.uk/spm). The first two scans of each session were discarded.
to allow for steady-state magnetization. The remaining images were realigned to the first image and spatially normalized to MNI stereotactic space using a standard EPI template as implemented in SPM2. The normalized images were spatially smoothed with a Gaussian kernel of 9 mm at full-width half-maximum. At the individual level, task-related changes in BOLD-signal were estimated at each voxel by modelling the time course of alternating blocks as delta functions convolved with a hemodynamic response function (HRF). Based on this model we computed a t-statistic for each voxel that tested for regional increases in BOLD signal during the finger sequence task. The result of the t-statistics was used to generate a SPM of task-related increases in BOLD signal. The contrast images obtained in each subject were entered into a two-sample t-test for between-groups comparisons to test for between-group differences in brain activations between mutation carriers and their respective control groups without mutation. The individual motor UPDRS scores were included in the analysis as covariate of no interest. The resulting t-values were corrected for multiple comparisons at voxel level, using the false discovery rate (FDR) correction method. Significance level was set at a corrected p-value of p<0.05. Any task-related BOLD signal change that reached an uncorrected p-value of 0.001 but failed to survive FDR correction is descriptively reported as statistical trend.

We defined the pre-SMA, PMd and intraparietal sulcus of both hemispheres as volumes of interest (VOI). The selection of cortical VOIs was based on previous neuroimaging studies. On the one hand, the pre-SMA and right PMd are increasingly active with the complexity of sequential finger movements in healthy individuals (Sadato et al., 1997, Boecker et al., 1998). On the other hand, patients with PD show bilateral increases in activity in PMd and intraparietal sulcus during sequential movements (Samuel et al., 1997, Catalan et al., 1999, Carbon and Eidelberg, 2006). Therefore, non-manifesting mutation carriers should show an additional recruitment of the pre-SMA. PMd and intraparietal sulcus during the finger sequence task. At the subcortical level, ROIs were placed in the posterior part of the putamen bilaterally. The putaminal ROIs were motivated by our recent morphometric study in which in a nearly identical group asymptomatic mutation carriers showed a putaminal increase in grey matter on T1-weighted structural MRIs (Weihofen et al., 2008). Each VOI was covered by a sphere. The diameter of the sphere was 27 mm which was threefold the FWHM used for Gaussian filtering. The spheres were centred on the peak increase in BOLD signal for the main effect of task. For these pre-defined VOIs, correction for multiple comparisons only considered voxels within the sphere. Outside the VOIs, all results were corrected across the whole brain.

Results

Behaviour. All participants found the thumb-to-finger opposition tasks easy to perform. The maximum error rate was two sequential errors per session. ANOVA revealed no difference in mean Tap1-Tap3 interval among groups (p>0.5). The mean Tap1-Tap3 interval was 1.44±0.18s among individuals carrying a Parkin mutation and 1.43±0.08s among controls without mutation. The mean Tap1-Tap3 interval was 1.40±0.11s in individuals with a PINK1 mutation and 1.36±0.08s in the corresponding controls.

Functional MRI. Epoch related analysis identified a bilateral set of sensorimotor areas where the BOLD signal increased when participants performed the finger sequence task (Fig. 1b and supplementary tables 1-3). Mutation carriers showed increased activation in right rostral PMd and the pre-SMA compared to controls (Fig. 2, Table 1). The overactivity in these rostral premotor areas was independent of the genotype (Fig. 2, Table 1). The pre-SMA and rostral PMd showed the most prominent increase in task related activation as compared to any other area in the brain. No additional activations emerged in any other brain area, even when we lowered the statistical threshold to an uncorrected P-value of 0.01 (extent threshold: 20 voxels). Mutation carriers showed no differences in task related deactivations relative to healthy controls. Relative to the corresponding control group, Parkin mutation carriers displayed an increased activation in the pre-SMA as well as a trend towards a stronger activation in the right rostral PMd. Likewise, PINK1 mutation carriers showed a bilateral overactivity in the pre-SMA which extended to the adjacent PMd. Controls without mutation showed no task-related regional increases in BOLD signal relative to the mutation carriers. The putamen showed a consistent task-related activation in all four groups. No between-group differences in task related activity were detected in the VOIs covering the right and left putamen. There were also differences in task-related BOLD signal changes between the two groups of mutation carriers. The left and right PMd as well as the anterior and medial portion of the left intraparietal sulcus displayed a stronger activation in PINK1 mutation carriers relative to Parkin mutation carriers (Fig. 3, Table 2). Additional trends towards an increased activation were observed in the right medial intraparietal sulcus, the anterior cingulate cortex, and left primary motor cortex. There was no relative increase in BOLD signal with sequential finger movements in Parkin as opposed to PINK1 mutation carriers.
Figure 2 Regional increases in task-related BOLD signal changes in non-manifesting carriers of a Parkin or PINK1 mutation. (A). Statistical parametric maps. Sagittal, coronal, and axial slices highlighting those voxels in the pre-SMA and adjacent PMd that showed a relative increase in BOLD signal during the sequential finger movement task in mutation carriers relative to controls without a mutation. The statistical parametric maps are superimposed on to a T2-weighted structural MRI template provided by MRicro (http://www.sph.sc.edu/comd/rorden/mricro.html). The voxels of the activation maps are colour-coded according to their Z-values. For illustrative purposes, the maps are thresholded at an uncorrected p-value of p<0.01.

Figure 1 Continued. (B) Parameter estimates of task-related BOLD signal changes in the right and left pre-SMA and right dorsomedial PMd. The column plots give the mean Beta-values (as estimated by the general linear model) for the task-related change in BOLD signal during the sequential finger movement task for each of the four groups (red columns = mutation carriers; yellow columns = non-mutation carriers). The Beta values are given in arbitrary units (A.U.) and refer to the voxel showing a peak difference between mutation carriers and non-carriers. Error bars equal the 95% confidence interval of the mean.

Table 1 Differences in task-related BOLD signal changes between mutation carriers of a Parkin or PINK1 mutation and healthy controls without mutation.

<table>
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Areas showing a relative increase in BOLD signal during the sequential finger movement in non-manifesting carriers. Differences in BOLD signal are characterized by their regional maxima (Z-score, P-value and x, y, z coordinates in MNI space). P-values are corrected for the number of voxels within the pre-defined spherical volumes of interest (for details see methods section). R = right; L = left; SVC = small volume correction; PMD = dorsal premotor cortex; SMA = supplementary motor area.
CHAPTER 4

Compensation in Parkin- and PINK1-Parkinsonism

The activation of the pre-SMA during sequential movements was attributed to the formation of and switch between visuo-motor associations rather than the control of the movements per se (Sakai et al., 1999, Rushworth et al., 2002). In the presence of a mutant Parkin or PINK1 allele, the “extra-recruitment” of the pre-SMA and adjacent PMd most likely reflects an adaptive mechanism by which the motor system counteracts the pre-existing latent nigrostriatal dysfunction (Hilker et al., 2001, Khan et al., 2002a, Khan et al., 2005).

Mutation carriers and controls showed equal performance when performing the simple motor sequence task. We propose that the mechanism by which mutation carriers maintain a normal level of performance is to recruit additional premotor regions that are specialized for handling complex sequential movements. We argue that the subclinical nigrostriatal neurotransmission in non-manifesting mutation carriers produced a dysfunction of the cortico-basal ganglia-thalamo-cortical motor loops involved in the control of overlearned motor sequences. This latent dysfunction rendered the simple sequence task more demanding in terms of

Discussion

When non-manifesting heterozygous carriers of a Parkin or PINK1 mutation perform a simple motor sequence task, they recruit the pre-SMA and right rostral PMd which are not utilized by healthy controls without mutation. This finding extends our recent morphometric MRI study showing an increase in grey matter volume in the basal ganglia in a comparable group of non-manifesting carriers of a Parkin or PINK1 mutation (Binkofski et al., 2007). Together, the functional and structural MRI data suggest that mutations in the Parkin and PINK1 gene produce a very similar functional and structural endophenotype. This implies that single heterozygous mutations in these two genes have a similar impact on the human motor system.

Converging evidence from neuroimaging and transcranial magnetic stimulation (TMS) show that in healthy individuals, the pre-SMA mainly contributes to motor sequence control in non-routine situations. Accordingly, functional neuroimaging demonstrated an activation of the pre-SMA and rostral right PMd with new or complex motor sequences but not with sequences that were highly overlearned or easy to perform (Sadato et al., 1997, Boecker et al., 1998, Sakai et al., 1999). The activation of the pre-SMA during sequential movements was attributed to the formation of and switch between visuo-motor associations rather than the control of the movements per se (Sakai et al., 1999, Rushworth et al., 2002). In the presence of a mutant Parkin or PINK1 allele, the “extra-recruitment” of the pre-SMA and adjacent PMd most likely reflects an adaptive mechanism by which the motor system counteracts the pre-existing latent nigrostriatal dysfunction (Hilker et al., 2001, Khan et al., 2002a, Khan et al., 2005).

Mutation carriers and controls showed equal performance when performing the simple motor sequence task. We propose that the mechanism by which mutation carriers maintain a normal level of performance is to recruit additional premotor regions that are specialized for handling complex sequential movements. We argue that the subclinical nigrostriatal neurotransmission in non-manifesting mutation carriers produced a dysfunction of the cortico-basal ganglia-thalamo-cortical motor loops involved in the control of overlearned motor sequences. This latent dysfunction rendered the simple sequence task more demanding in terms of

Table 2 Differences in task-related BOLD signal changes between carriers of a single mutant PINK1 or Parkin allele.

<table>
<thead>
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Areas showing a relative differences in task-related BOLD signal changes between the two groups of non-manifesting mutation carriers. Differences in BOLD signal are characterized by their regional maxima (Z-score, P-value and x, y, z coordinates in MNI space). P-values are corrected for the number of voxels within the pre-defined spherical volumes of interest (for details see methods section). R = right; L = left; SVC = small volume correction; PMD = dorsal premotor cortex; CMA = cingulate motor area; M1_hand = primary motor hand area; IPS = intraparietal sulcus.)

Figure 3 Relative increases in task-related BOLD signal changes in non-manifesting carriers of a PINK1 mutation compared with non-manifesting carriers of a Parkin mutation.

Axial slices showing voxels in dorsal frontoparietal cortex with significant increase in BOLD signal during the sequential finger movement task in PINK1 mutation carriers relative to Parkin mutation carriers. The statistical parametric maps are superimposed on to a T2-weighted structural MRI images provided by MRicro (http://www.sph.sc.edu/comd/rorden/mricro.html). The voxels of the activation maps are colour-coded according to their Z-values. For illustrative purposes, the maps are thresholded at an uncorrected p-value of p<0.01.

4
neuronal motor control and specifically called on the “support” of rostromedial premotor cortex to maintain task performance. (Isoda and Hikosaka, 2007) Because the pre-SMA and rostromedial PMd are reciprocally connected with prefrontal areas (Luppino et al., 1993, Lu et al., 1994) and receive inputs from the “non-motor” (associative) territories of the cerebellum and basal ganglia (Akkal et al., 2007), the increased activation of the pre-SMA and rostral PMd might be driven by a compensatory increase in neuronal input from connected prefrontal or subcortical areas during the task.

The adaptive recruitment of cortical premotor areas was restricted to the pre-SMA and the dorsomedial part of right rostral PMd. In a previous fMRI study, we also found an increase in rostral motor areas in non-manifesting carriers of a Parkin mutation, but the spatial pattern of increased activity was different from the one found in the present study. When participants selected a finger movement with their right hand based on internal cues, individuals with a mutant Parkin allele showed a stronger activation of the rostral cingulate motor area and the ventrolateral part of left rostral PMd but not in rostral SMA and right rostromedial PMd (Buhmann et al., 2005). These findings lend support to the notion that a latent nigrostriatal dopaminergic dysfunction gives rise to variable patterns of activity changes in rostral premotor regions which critically depend on the specific motor functions probed by the experimental task. The observation that the compensatory recruitment of cortical motor areas is task-specific underscores the capacity of human sensorimotor networks to flexibly adapt to a regional dysfunction (Lee et al., 2003).

We also identified some differences between carriers of mutations in the Parkin or PINK1 gene. PINK1 mutation carriers, we observed an additional mainly left-hemispheric recruitment of frontoparietal areas, including distinct areas in left caudal PMd and intraparietal sulcus. It is unclear whether this reflects a true genotype-specific pattern of functional adaptation if the frontoparietal cortex. One possibility is that non-manifesting PINK1 mutation carriers have a stronger functional impairment of the motor system, requiring recruitment of additional frontoparietal loops. Future studies could resolve this issue by correlating adaptive redistribution of cortical activity to the depth of the nigrostriatal dopaminergic deficit, for example using nuclear imaging.

An intriguing question is whether these increases in task-related activity persist, increase or attenuate in mutation carriers who ultimately develop PD. A recent H$_2$O PET study provided some evidence that the extra-recruitment of frontal motor areas still represents an effective mechanism of compensation in the early stage of sporadic PD (Mentis et al., 2003). In that study, PD patients achieved equal performance with healthy controls when learning short motor sequences. In patients, equal performance was associated with the additional recruitment of cortical areas that are normally further address this important question specialized for learning more difficult sequences. Additional MRI studies on high-risk populations as well as cross-sectional studies on drug-naive patients with newly diagnosed sporadic or monogenic PD are needed to.

Acknowledgements
This work has been supported by a BMBF grant to H.R.S. (01 GO 0511) and F.B. (01 GO 0512) (NeuroImage-Nord) and by the 6th European Framework (EU-LSHB-CT-2006-037544 - GENEPARK). C.K., F.B., and H.S. have been supported by the Volkswagenstiftung. B.F.L.v.N. and B.R.B. were supported by a NWO VIDI research grant (number: 917.76.352).
Supplementary table 1

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MNI stereotactic coordinates of local maxima of motor regions showing main effect of task in control subject without mutation in the Parkin or PINK1 gene. For large clusters spanning several anatomical regions more than one maximum is given. The intensity threshold was set at P < 0.05 (FWE corrected). M1: primary motor cortex; PMv: ventral premotor cortex; PMd: dorsal premotor cortex; aIPS: anterior Intraparietal sulcus; SMA: supplementary motor area; CMA: cingulate motor area.

Supplementary table 2

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MNI stereotactic coordinates of local maxima of motor regions showing main effect of task in carriers of a single mutant Parkin allele. For large clusters covering several anatomical regions more than one regional maximum is given. The intensity threshold was set at P < 0.05 (FWE corrected). M1: primary motor cortex; PMv: ventral premotor cortex; PMd: dorsal premotor cortex; aIPS: anterior Intraparietal sulcus; SMA: supplementary motor area; SMG: supramarginal gyrus; CMA: Cingulate motor area.
CHAPTER 4

COMPENSATION IN PARKIN- AND PINK1-PARKINSONISM

References


Supplementary table 3

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<tr>
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<tr>
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<td>L</td>
<td>-38 -62 -32</td>
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MNI stereocordinates of local maxima of motor regions showing main effect of task in carriers of a single mutant PARK1 allele. For large clusters spanning several anatomical regions more than one regional maximum is given. The intensity threshold was set at P < 0.05 (FWE corrected). M1: primary motor cortex; PMv: ventral premotor cortex; PMd: dorsal premotor cortex; aIPS: anterior Intraparietal sulcus; SMA: supplementary motor area; CMA: cingulate motor area.


Cerebral pathological and compensatory mechanisms in the premotor phase of LRRK2 parkinsonism

BFL. van Nuenen, RC Helmich, M Ferraye, A Thaler, T Hendler, A Orr-Urtridge, A Mirelman, S Bressman, KS Marder, N Giladi, BPC van de Warrenburg, BR Bloem and I Toni on behalf of the LRRK2 Ashkenazi Jewish Consortium

Brain; in press

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Abstract

Compensatory cerebral mechanisms can delay motor symptom onset in Parkinson’s disease. We aim to characterize those compensatory mechanisms, as well as early disease-related changes, by quantifying movement-related cerebral function in subjects at significantly increased risk to develop Parkinson’s disease, namely carriers of a leucine-rich repeat kinase 2-G2019S mutation associated with dominantly inherited parkinsonism. Functional magnetic resonance imaging was used to examine cerebral activity evoked during internal selection of motor representations, a core motor deficit in clinically overt Parkinson’s disease. Thirty-nine healthy first-degree relatives of Ashkenazi Jewish Parkinson’s disease patients that carry the leucine-rich repeat kinase 2-G2019S mutation participated in this study. Twenty-one carriers of the leucine-rich repeat kinase 2-G2019S mutation and 18 non-carriers of this mutation were engaged in a motor imagery task (laterality judgments of left or right hands) known to be sensitive to motor control parameters. Behavioural performance of both groups was matched. Mutation carriers and non-carriers were equally sensitive to the extent and biomechanical constraints of the imagined movements in relation to the current posture of the participants’ hands. Cerebral activity differed between groups, such that leucine-rich repeat kinase 2-G2019S carriers had reduced imagery-related activity in the right caudate nucleus and increased activity in the right dorsal premotor cortex. More severe striatal impairment was associated with stronger effective connectivity between the right dorsal premotor cortex and the right extrastriate body area. These findings suggest that altered movement-related activity in the caudate nuclei of leucine-rich repeat kinase 2-G2019S carriers might remain behaviorally latent by virtue of cortical compensatory mechanisms involving long-range connectivity between the dorsal premotor cortex and posterior sensory regions. These functional cerebral changes open the possibility to use a prospective study to test their relevance as early markers of Parkinson’s disease.

Introduction

Clinical symptoms of Parkinson’s disease (PD) emerge when cerebral degenerative processes overcome compensatory mechanisms. Both phenomena start several years before clinical onset of PD (Falop et al., 2006), but their exact cerebral correlates at the system level are unknown. Experimentally induced dopaminergic depletion in animals treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) provides a controlled window into the presymptomatic period of induced parkinsonism. These studies revealed sensory-driven compensatory mechanisms in cortico-thalamo-cortical circuits (Bezard et al., 2003, Escola et al., 2003, Pessiglione et al., 2003), but it remains unclear to what extent these animal models can be generalized to human PD. More recently, neuroimaging studies in non-manifesting carriers of mutations known to cause monogenetic forms of PD, like Parkin (PARK2) or PINK1 (PARK6) mutations (Broussolle et al., 2000, Hilker et al., 2001, Khan et al., 2002a,b, Khan et al., 2002b, Walter et al., 2004, Buhmann et al., 2005, Khan et al., 2005b, Baumer et al., 2007, Binkofski et al., 2007, Hagenah et al., 2007, Schweitzer et al., 2007, Hagenah et al., 2008, van Nuenen et al., 2009a, van Nuenen et al., 2009b, Reetz et al., 2010, Saunders-Pullman et al., 2010), have been used to map cerebral changes occurring before the clinical onset of PD (Farrer, 2006). Non-manifesting carriers of these mutations showed structural and functional alterations similar to those observed in idiopathic PD, e.g. nigrostriatal and premotor dysfunctions. However, it remains unclear whether the recessive genetic markers studied so far are representatives of classic PD phenotypes, particularly for heterozygous carriers of mutations in recessive genes. For example, neuropathological examination of PD patients with homozygous and compound heterozygous Parkin mutations have demonstrated absence of Lewy bodies, questioning whether the pathophysiological mechanism in Parkin-related PD might be different from idiopathic PD (Pouliaopoulos et al., 2012). Also, there is imaging evidence to suggest that different genetic mutations lead to different cerebral phenotypes: non-manifesting Parkin, PINK1 and ATP13A2 mutation carriers have increased grey matter volume in the putamen, whereas leucine-rich repeat kinase 2 (LRRK2) carriers have increased grey matter volume in the right caudate nucleus (Reetz et al., 2010).

Here we study carriers of the LRRK2 mutation, the most frequent genetic cause of PD worldwide (Farrer, 2006). This dominantly inherited risk factor might be associated with changes in brain activity reflecting pathological and compensatory mechanisms of PD before symptoms become clinically evident. We characterize functional and structural markers of cortical changes associated with the LRRK2 G2019S mutation, comparing asymptomatic LRRK2 carriers with non-mutation carriers. We assess a core deficit of PD: the internal selection of motor representations
(Brown and Marsden, 1988, Helmich et al., 2009). To identify these markers, we use a validated motor imagery task in which subjects are asked to make laterality judgments (left or right) of hand pictures (de Lange et al., 2005, Helmich et al., 2009), while measuring behavioral performance (reaction times) and cerebral activity (using fMRI). Previous studies have shown that subjects solve this task by mentally moving their own hand from its current position into the stimulus orientation for comparison (Parsons, 1987, de Lange et al., 2008a). This task allows subjects to mentally select which hand is going to be mentally moved, according to hand-specific biomechanical constraints and the subjects’ own hand posture (de Lange et al., 2006). The validity of this task has been demonstrated both in healthy subjects (Shenton et al., 2004, de Lange et al., 2006) and in PD patients (Dominey et al., 1995, Helmich et al., 2007, Helmich et al., 2011). The known pathophysiology of LRRK2 suggests that latent dopaminergic deficits in LRRK2 mutation carriers might result in impaired activity in the striatum (Galter et al., 2006, Lin et al., 2011). We also predict that, similar to what has been observed in idiopathic PD patients (Helmich et al., 2007, van Nuenen et al., 2012), increased connectivity between posterior sensory areas and the cortical motor system might compensate for those striatal deficits.

Methods

We included 62 first-degree asymptomatic first-degree relatives of Ashkenazi patients with Parkinson’s disease who are carriers of the G2019S mutation in the LRRK2 gene (age 47.2±9.7 years, mean±S.D.; 28 men). Given the dominant inheritance of this mutation, approximately 50% of the subjects also have the mutation and are at higher risk to develop PD. All subjects were native Hebrew speakers and provided written informed consent after receiving full explanation on the nature of the study. The study was approved by the Tel Aviv Sourasky Medical Center Institutional Review Boards. Before scanning, subjects underwent a complete neurological examination, including the Unified Parkinson’s Disease Rating Scale (UPDRS).

Genomic DNA was isolated from peripheral blood using standard protocols or from saliva according to manufacturer’s instructions (Oragene, Ottawa, Canada). To detect the 6055G_A (G2019S) mutation (rs34637584) in LRRK2 exon 41, we amplified a 171 bp fragment with the following primers: forward 5’ CCTGTGCTTTTCTGGCAGATA 3’ and reverse 5’ CCTCTGATGTTTTTATCCCCATTC 3’ as previously described (Orr-Urtreger et al., 2007). PCR fragments were sequenced using the BigDye Terminator Chemistry (Applied Biosystems, Foster City, CA) and analyzed using an automated ABI Prism 3130x1 Genetic Analyzer (Applied Biosystems). In addition LRRK2 G2019S mutation was also detected using TaqMan assay ID C_63498123_10 in the StepOnePlus Real-Time PCR System (Applied Biosystems). The participants did not know their genotype status.

Exclusion criteria were: clinical diagnosis of PD according to the Queen Square Brain Bank Criteria (Gibb and Lees, 1988), other neurological diseases (such as severe head trauma, stroke or history of psychiatric disease treated with neuroleptics), general exclusion criteria for MRI scanning (such as claustrophobia, pace-maker, and implanted metal parts), and failure to perform the motor imagery task during a training session outside the scanner (error rate >90%), or inside the scanner (error rate >30%). We also excluded subjects with severe head movements (Euclidean distance >4.0 mm). During data collection both examiners and participants were blinded for the mutation status.

Motor Imagery Experiment

We used line drawings of left and right hands, with either the back or palm of the hand in view (figure 1). The left and right hand drawings were identical mirror images. The hand drawing could be rotated in either a counter-clockwise or a clockwise orientation. For both orientations, four different rotations (45°, 75°, 105° and 135°) were used; this yielded eight different rotations. These stimuli were presented through a PC running Presentation software (Neurobehavioural systems, Albany, USA). Images were projected via an LCD projector (NEC, VT660K) onto a screen positioned in front of the subjects’ forehead and viewed through a tilted mirror. Responses were gathered with an MRI-compatible response box (HH-1 × 4L, Current Designs) and saved on a log-file for further analysis. Prior to entering the fMRI, all participants underwent a preparatory session during which adequate performance of the task was assured. Inside the scanner, participants were asked to report whether the drawing of the hand on display represented a left or a right hand (regardless of its rotation) by pressing one of the buttons that were located underneath their left and right big toes. During scanning, reaction times and error rates were measured for subsequent behavioral analysis. The imaging session consisted of 32 task blocks (duration 60 s per block) intermixed with 30 baseline periods (duration 10 s). Each block consisted of 12 trials, which started with a fixation cross, displayed for a variable interval (0.5–1.5 s), followed by the presentation of a drawing of a hand. When a response was provided, the stimulus was replaced by the fixation cross for a jittered period of 1.5-2.5 s and then a subsequent drawing was shown. Subjects did not receive feedback. Rotation and laterality of the hand drawings were randomized from trial to trial. On the basis of previous studies (Helmich et al., 2007), the RT cut-off was set at 5.0 s. In total, subjects performed 384 trials. During the experiment, the posture of the patients’ left and right hand was manipulated. At the beginning of each block, a text instructed
CHAPTER 5

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the effects of biomechanical constraints and body posture on behavioral performance. The term “biomechanical constraints” (BMC) refers to the reaction time difference in mentally rotating a hand towards a lateral, as compared to a medial orientation with respect to the body axis. Lateral and medial orientations were coded as follows: counter-clockwise rotations (-135°, -105°, -75°, -45°) were averaged and recoded as a lateral orientation for left hands and a medial orientation for right hands; clockwise rotations (45°, 75°, 105°, 135°) were averaged and recoded as a medial orientation for left hands and a lateral orientation for right hands. The term “hand posture” refers to the posture of the participants’ hand (pronated or supinated) with respect to the posture of the hand drawing (irrespective of medio-lateral rotation). Trials were coded as matching (MATCH) or non-matching (NONMATCH) with respect to the subjects’ own hand posture. Thus, we tested the effects of factors HAND (2 levels: LEFT or RIGHT), BMC (2 levels: MEDIAL or LATERAL), hand posture (2 levels: MATCH or NONMATCH) and GROUP (2 levels: non-mutation carriers or nonmanifesting LRRK2-carriers) by means of repeated measures ANOVA on RTs collected during scanning. The Greenhouse–Geisser method was used to correct for non-sphericity. Alpha-level was set at \( p = 0.05 \).

Functional MRI image acquisition and preprocessing

Imaging was performed on a GE 3T Signa HDxt scanner with a resonant gradient echoplanar imaging system. All images were acquired using a standard 8-channel head coil. Each subject received an anatomical scan using spoiled gradient (3D-SPGR), echo sequence with field of view (FOV) 250×250 mm; matrix size 256×256; voxel size 0.98×0.98×1; Repetition Time (TR) 9; Echo Time (TE) 3.6 ms and functional scans (64x64, FOV 20, 35 slices, 3.5 thickness, no gap, TR 2200, REP 57). Functional data were pre-processed and analyzed with SPM5 (Statistical Parametric Mapping, http://www.fil.ion.ucl.ac.uk/spm). First, functional EPI images were spatially realigned using a least squares approach and a six parameter (rigid body) spatial transformation (Friston et al., 1995). Subsequently, the time-series of each voxel was realigned temporally to acquisition of the first slice (slice time correction). Anatomical images were spatially co-registered to the mean of the functional images (Ashburner and Friston, 1997) and segmented using a unified segmentation approach. The resulting transformation matrix was then used to normalize the anatomical and functional images. The normalized functional images were re-sampled at an isotropic voxel size of 2 mm and smoothed with an isotropic 8 mm full-width-at-half-maximum (FWHM) Gaussian kernel.

Analysis of task-related effects

First-level analysis: The pre-processed fMRI time series were analyzed at the first level using an event-related approach in the context of the general linear model...
carriers, we next hypothesized a change in connectivity between this
p
carriers may also have increased connectivity between
°; PARKINSONISM
°). In addition, our first-level
COMPENSATION IN
et al., 2006, Helmich et al., 2007). We localized the left and right PMd by testing for
searched for differential imagery-related activity in the PMd, because this region is
known to be specifically involved in motor imagery of hand movements (de Lange
et al., 2006, Helmich et al., 2007). We localized the left and right PMd by testing for
rotation-related activity across the whole group (i.e. in both carriers and non-mutation
 carriers), correcting for multiple comparisons across the whole brain. This analysis

We also performed region of interest (ROI) analyses, using a voxel-level statistical
threshold of \( p < 0.01 \) family-wise error (FWE) corrected for multiple comparisons
over the search volume (i.e. the ROI). There were seven regions of interest. First we
searched for differential imagery-related activity in the PMd, because this region is
known to be specifically involved in motor imagery of hand movements (de Lange
et al., 2006, Helmich et al., 2007). We localized the left and right PMd by testing for
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\text{rotation-related activity across the whole group (i.e. in both carriers and non-mutation
\text{ carriers), correcting for multiple comparisons across the whole brain. This analysis
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Voxel-based morphometry

We considered the possibility that between-groups differences in functional activity could reflect between-groups anatomical differences. Therefore we performed a voxel-based morphometry (VBM) analysis. VBM analyses were done in SPM8. We segmented the anatomical MRI scan of each subject into gray matter, white matter, cerebrospinal fluid, and extra-cerebral compartments (e.g. out-of-brain, skull, skin). We used the DARTEL toolbox (Ashburner, 2007) as implemented in SPM8 to create a study-specific anatomical template and register all individual gray matter images to this template. All images were subsequently normalized to MNI space, while correcting for volume changes induced by normalization. Last, we smoothed all gray matter images using a kernel of 10 mm FWHM, and we performed a regression analysis on these smoothed images to test for differences in gray matter between the two groups (non-mutation carriers and nonmanifesting LRRK2-carriers). We also included age, gender, as well as total gray matter as covariates, since these factors have been shown to have a great impact on gray matter volume (Good et al., 2001). To maximize the power of the VBM analysis, we also included the subjects who were not able to adequately perform the fMRI motor imagery task. Therefore the VBM analysis included in total 28 non-mutation carriers and 34 nonmanifesting LRRK2-carriers. Besides whole-brain analysis, we focused the inferences on a set of ROIs based on previous VBM studies on nonmanifesting PARK-gene carriers (Binkofski et al., 2007; Reetz et al., 2010). We used an automated parcellation method [AAL atlas, (Tzourio-Mazoyer et al., 2002)] as implemented in the WFU-Pickatlas SPM8 toolbox (Maldjian et al., 2003), to generate anatomical masks on putamen and caudate nuclei in each hemisphere for each subject.

Results

Participants

Of the 62 included candidates, 34 (55%) had the G2019S LRRK2 mutation. Thirteen subjects had an error rate higher than 30% and were excluded from further analysis. It remains unclear why these participants had a poor task performance, but it seems unlikely to be related to their genetic status and any ongoing cerebral alteration. First, although a larger number of mutation carriers (eight) were excluded than non-mutation carriers (five), the proportion of excluded subjects is similar across the two groups ($X^2=2$, $p = 0.157$). Second, previous work (Helmich et al., 2007; van Nuenen et al., 2012) clearly indicates that even patients at quite advanced stages of PD (mean H&Y 2.1 and 1.4 respectively; range 1-3) can effectively perform the imagery task used in this study. However, in the above mentioned studies, participants and patients were trained before scanning to perform with an error rate < 10%, whereas in this study participants had a much shorter training. The reason for limited training in this study is that these participants performed an extensive battery of tasks (Pen and paper questionnaires: Montreal Cognitive Assessment (MoCa), University of Pennsylvania Smell Identification Test (UPSIT), State-Trait Anxiety Inventory (STAI), Blessed Dementia Scale (BDS), Trail Making Test (TMT), The Unified Parkinson’s Disease Rating Scale (UPDRS), Category Verbal Fluency Test, The Scale for Outcomes in Parkinson’s disease for Autonomic Symptoms (SCOPA-AUT), Digit Span Test, The Stroop color-word test, Finger tapping. Computerized test: Neurotrax - a computerized cognitive program (Thaler et al., 2012). Motor test: Gait performance across 4 different walking velocities. In the MR scanner, the following tasks and imaging procedures were acquired: Motor imagery task (fMRI, reported in this manuscript), DTI scan, Resting state (fMRI), Observation of 2 movie clips (fMRI), Stroop task (fMRI), n-back task (fMRI), Domino task (fMRI), Structural scan (data used in this manuscript) lasting over three hours. One subject was excluded because of claustrophobia, three subjects were excluded because of technical problems and five subjects were excluded because of movement artifacts. One subject had a mutation in the glucocerebrosidase gene (GBA), and was excluded because we were specifically interested in LRRK2 mutation carriers because they have an increased risk for developing PD. GBA may or may not influence the risk for developing PD. To keep our comparison between LRRK2 carriers and non-carriers as clean as possible, we decided to exclude the subject with both LRRK2 and GBA mutation. Of the remaining 39 subjects, the LRRK2 G2019S mutation screening revealed a mutation in 21 participants (54%; 11 men; 48 ± 9 years (mean ± S.D.)) and no LRRK2 G2019S mutation in 18 participants (46%; 8 men; 45 ± 9 years). There were no significant baseline differences between the two groups (Table 1).

Table 1 Groups characteristics

<table>
<thead>
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<th>Non-mutation carriers</th>
<th>Asymptomatic LRRK2-carriers</th>
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<td></td>
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<td>47.6 ± 9.1</td>
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<tr>
<td>gender (%men)</td>
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<td>57.1</td>
<td></td>
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<tr>
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<td>20 RH / 1 LH</td>
<td></td>
</tr>
<tr>
<td>UPDRS III</td>
<td>1.8 ± 1.3</td>
<td>1.9 ± 1.7</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Baseline characteristics of subjects included in the fMRI experiment.

RH = right handedness; LH = left handedness; UPDRS = Unified Parkinson’s Disease Rating Scale; Values indicate mean ± S.D.
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COMPENSATION IN LRRK2-PARKINSONISM

Behavioural results
The mean error rate was comparable between groups (non-mutation carriers: 10 ± 2%; LRRK2 carriers: 10 ± 2%; t(37) = -0.123, p = 0.913; mean ± S.E.M.) as were the mean reaction times (non-mutation carriers: 1418 ± 86 ms; LRRK2 carriers: 1443 ± 96 ms; t(37) = -0.192, p = 0.849). The reaction times increased with increasing rotation of the hand drawing (main effect of ROTATION: F(3,46)=53.15; p<0.001). This effect was not influenced by the mutation carrier status (ROTATION x GROUP interaction: F(7,59)=1.93; p=0.124), or by the hand used to perform motor imagery (HAND X ROTATION x GROUP interaction: F(7,64)=0.56; p=0.683; See Fig 2).

We then tested for the effects of stimulus orientation (biomechanical complexity, BMC: difficult or easy) and the effect of the subjects’ own body posture (matching or non-matching with respect to the stimulus) on reaction times. Reaction times were longer for stimuli in a biomechanically difficult than easy orientation (F(1,17)=62.21; p<0.001), and they were longer for stimuli that did not match the posture of the subjects’ own hand (POSTURE: F(1,17)=15.99; p=0.001). The subjects were faster when the stimuli were right hands (HAND: F(1,17)=9.35; p=0.007), and the behavioral slowing induced by the biomechanical complexity of the stimulus was larger for right than for left hands (HAND X BMC: F(1,17)=6.25; p=0.023). Importantly, there were no significant interactions with the factor GROUP (all p>0.2). These results strongly suggest that both groups used first-person motor imagery to solve the task (i.e. they mentally rotated their own hands, taking into account their current posture and biomechanical constraints of the imagined movements), rather than visual imagery. Having used a task that LRRK2 carriers can solve as well as non-mutation carriers, it becomes meaningful to compare cerebral responses between groups during performance of correct trials (Price and Friston, 1999).

Imaging data
Rotation-related effects
First, we identified regions where activity increased with increasing stimulus rotation. We found a bilateral parieto-premotor network, confirming its involvement in mental rotation of hands (table 2) (Johnson et al., 2002, de Lange et al., 2006, Helmich et al., 2007). Second, we tested for differential rotation-related changes in cerebral activity between LRRK2 carriers and non-mutation carriers (GROUP X ROTATION interaction). The head of the left caudate showed increased activity during increasing stimulus rotation in non-mutation carriers compared to LRRK2 carriers (MNI coordinates [-16 -2 +20]; z = 4.36; p = 0.006 corrected), and we observed a trend for the same effect in head of the right caudate (MNI coordinates [+18 +2 +18]; z = 3.66; p = 0.063 corrected). The right PMd showed increased activity during increasing stimulus rotation in LRRK2 carriers compared to non-mutation carriers (MNI coordinates [34 -12 +46]; z = 3.24; p = 0.040 corrected; Figure 2A).
Biomechanical constraints and posture
As described above, reaction times were longer when the imagery task involved laterally-oriented hands. Cerebral activity following the same pattern was found in the bilateral insula, posterior parietal cortex, and middle occipital gyrus (table 3). There were no between-group differences in cerebral activity related to biomechanical complexity. At the conservative statistical threshold used in this study, we did not find significant posture-related cerebral activity, and no posture-related differences between groups.

Effective connectivity
From the contrasts mentioned above, it emerged that the right PMd was more active during increasing stimulus orientation in LRRK2 carriers than non-mutation carriers. Building on recent observations in symptomatic PD patients, obtained during performance of the same task used in this study, we hypothesized that the increased PMd activity might relate to increased connectivity with the EBA (Helmich et al., 2007). We tested this hypothesis with PPI, a tool designed to assess changes in effective connectivity between cerebral regions (Friston et al., 1997). Using the right PMd as the seed region and the right EBA as the target region, we found that functional connectivity between these regions increased with stimulus rotation in LRRK2 carriers but not in non-mutation carriers (effect of GROUP: MNI coordinates [+40 -78 +4]; t = 3.71; p = 0.032 corrected; figure 3B). In a whole brain analysis without the right EBA as a target region, no brain area reached significance (p<0.05, corrected). Next, we reasoned that if this increased connectivity compensates for latent dopaminergic dysfunction in the striatum, then connectivity should increase as task-related activity in the right caudate decreases. To test this, we correlated task-related activity in the right caudate (beta values at [+18 +2 +18] from rotation-related contrast image) with PMd-EBA connectivity (beta values at [+40 -78 +4] from PPI contrast image) separately for LRRK2 carriers and non-mutation carriers. In LRRK2 carriers, reduced activity in the right caudate predicted increased PMd-EBA connectivity (r = -0.580; p = 0.018). There was no significant correlation for the non-mutation carriers (r = 0.101; p = 0.731; Figure 3C).

Voxel-based morphometry
There were no significant differences in gray matter volume between groups, even when lowering the statistical threshold to a lenient threshold of p<0.01 uncorrected, and even when restricting our search to the striatum or to brain areas showing differential imagery-related activity. This indicates that the fMRI findings we report are not a by-product of substantial structural changes in LRRK2 carriers.
## Table 3  Cerebral activity related to biomechanical constraints

<table>
<thead>
<tr>
<th>Anatomical region</th>
<th>Hemisphere</th>
<th>Functional region</th>
<th>Local maximum</th>
<th>Cluster size</th>
<th>p-value</th>
<th>Cluster t-value</th>
<th>t-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Middle occipital gyrus V2</td>
<td>L</td>
<td>PPC</td>
<td>-14</td>
<td>34</td>
<td>0.001</td>
<td>-2</td>
<td>3.38</td>
</tr>
<tr>
<td>Dorsal intraparietal sulcus V2</td>
<td>R</td>
<td>PPC</td>
<td>16</td>
<td>-62</td>
<td>0.001</td>
<td>-2</td>
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<td>R</td>
<td>PPC</td>
<td>16</td>
<td>-62</td>
<td>0.001</td>
<td>-2</td>
<td>3.38</td>
</tr>
<tr>
<td>Insular cortex</td>
<td>L</td>
<td>PPC</td>
<td>36</td>
<td>-48</td>
<td>0.021</td>
<td>-2</td>
<td>3.22</td>
</tr>
<tr>
<td>Inferior parietal lobe</td>
<td>L</td>
<td>PPC</td>
<td>36</td>
<td>-48</td>
<td>0.021</td>
<td>-2</td>
<td>3.22</td>
</tr>
</tbody>
</table>

- **Anatomical region**
  - Middle occipital gyrus
  - Dorsal intraparietal sulcus
  - Insular cortex
  - Inferior parietal lobe
- **Hemisphere**
  - L: Left
  - R: Right
- **Functional region**
  - PPC: Posterior parietal cortex
  - V2: Visual area 2
- **Local maximum**
  - t-value for cluster size
- **Cluster size**
  - in number of voxels
- **p-value**
  - <0.05 for statistical inference
- **Cluster t-value**
  - uncorrected at cluster level
- **t-value**
  - threshold necessary to determine the cluster-level threshold

### Figure 3 Cerebral effects.

**A**: Between-groups differences in imagery-related brain activity. Anatomical distribution of voxels that showed a differential change in BOLD signal as a function of stimulus rotation (i.e., imagery-related) between non-mutation carriers (NMC) and non-manifesting LRRK2 mutation carriers (MC). On the left, axial slice showing reduced imagery-related BOLD signal in the caudate nuclei; On the right, axial slice showing increased imagery-related BOLD signal in the dorsal premotor cortex (PMd). Statistical parametric maps (SPMs) of imagery-related between-groups differences (Z-scores, in red-yellow) superimposed onto a T2-weighted structural MRI template (in grey). For illustrative purposes, SPMs are thresholded at an uncorrected p value of p<0.01 with a cluster extent threshold of > 50. **B**: Between-groups differences in imagery-related effective connectivity. The coronal slice on the right describes the anatomical distribution of voxels in the extrastriate body area (EBA) with differential increase in imagery-related coupling with the right PMd (left axial slice) between non-mutation carriers and non-manifesting LRRK2 mutation carriers. The SPM(Z) of between-groups differences in PMd-EBA imagery-related connectivity (in red-yellow) is superimposed onto a T2-weighted structural MRI template (in grey). **C**: Between-groups differences in the relation between imagery-related activity in the right caudate and PMd-EBA connectivity. Scatterplot of parameter estimates (ß-values) of imagery-related activity in the right caudate (x = 18, y = 2, z = 18) against coupling strength between the right PMd (x = 34, y = -12, z = 46) and the right EBA (x = 40, y = -78, z = 4) for non-mutation carriers (left panel) and for non-manifesting LRRK2 mutation carriers (right panel).
Discussion

We studied a large group of asymptomatic LRRK2-G2019S mutation carriers to identify potential preclinical cerebral reorganisation during a motor imagery task requiring internal selection of motor representations (de Lange et al., 2006, Helmich et al., 2007). This task was selected because it captures a core dysfunction in clinical stages of PD (Brown and Marsden, 1986, Helmich et al., 2009). There are three main results. First, LRRK2-G2019S mutation carriers and non-carriers were equally sensitive to the extent and biomechanical constraints of the imagined movements, in relation to the current posture of the participants’ hands. This result confirms that this motor imagery task relies on internal selection of motor representations (de Lange et al., 2008a). This result also indicates that the observed cerebral effects were not driven by between-groups differences in performance. Second, asymptomatic LRRK2-G2019S mutation carriers had reduced imagery-related activity in the right caudate nucleus. This result indicates that asymptomatic LRRK2-G2019S mutation carriers have a functional impairment in the striatum. Furthermore, in contrast to the structural alterations seen in the ventrolateral striatum of idiopathic PD patients and non-manifesting carriers of Parkin and PINK1 mutations (Buhmann et al., 2005, Binkofski et al., 2007, Reetz et al., 2008), the striatal impairment in asymptomatic LRRK2 mutation carriers arose in the caudate nucleus. Third, asymptomatic LRRK2-G2019S mutation carriers had increased imagery-related activity in the right PMd. Effective connectivity between this region and the right extrastriate body area increased in proportion to the caudate alteration. This suggests that long-range connectivity between the PMd and posterior sensory regions might compensate for striatal impairments, a mechanism recently observed in idiopathic PD patients (Helmich et al., 2007, van Nuenen et al., 2012).

Altered caudate functionality in the premotor phase of LRRK2 parkinsonism

The motor imagery task evoked largely overlapping cerebral responses across LRRK2 mutation carriers and non-mutation carriers, namely activity in parieto-fron- tal regions previously associated with performance of this task (de Lange et al., 2006, Helmich et al., 2007). This result indicates that the two groups used similar cerebral circuits for solving the motor imagery task. A notable exception was found for the head of the caudate nucleus, showing less activity in non-manifesting LRRK2 mutation carriers. Given that the premotor phase of LRRK2 parkinsonism is also accompanied by nigrostriatal dopaminergic deficits (Khan et al., 2005a, Bruggemann et al., 2011), the imagery-related difference observed in the caudate might constitute a functional correlate of that dopaminergic deficit. Yet, this observation does not fit with the known consequences of dopaminergic denervation in symptomatic PD patients, namely earlier and stronger alterations of the putamen than the caudate nucleus (Kish et al., 1988). However, if non-manifesting LRRK2 carriers have a similar upregulation of the nigro-striatal dopamine system as observed in non-manifesting MPTP-treated monkeys (Mounayar et al., 2007), then dopamine levels of non-manifesting LRRK2 carriers could be normalized in the putamen, but overdosed in the relatively less depleted caudate. Given the restricted physiological range of dopaminergic modulation (Goldman-Rakic et al., 2000), an excess of dopamine in the caudate could lead to increased lability of the motor representations relevant for the imagery task (Cools and D’Esposito, 2011). The reduced caudate contributions observed in non-manifesting LRRK2 mutation carriers could then reflect a reduced ability of this striatal structure to support temp- orally-sustained movement representations as required by the current motor imagery task. Given the known involvement of the head of the caudate in executive functions (Alexander et al., 1986, Marklund et al., 2009), the present finding would predict that LRRK2 carriers are characterized by cognitive alterations early in the disease, although this effect might be also driven by non-dopaminergic alterations (Alcalay et al., 2012).

A compensatory role for the dorsal premotor cortex?

Non-manifesting LRRK2 mutation carriers and non-mutation carriers solved the motor imagery task equally well, but the former group had stronger activity in the right PMd. This effect is clearly located within the probabilistic cytoarchitectonic borders of Brodmann area 6 (Eickhoff et al., 2005), and more precisely within the dorsal premotor cortex (Mayka et al., 2006; Tomassini et al., 2007). On the basis of the guidelines offered by Picard & Strick (Picard and Strick, 2001), we infer that the current effect is located in the caudal sector of the dorsal premotor cortex, being about 8 mm anterior to the primary motor cortex (as inferred on the basis of Mayka et al, Neuroimage 2006). This portion of the dorsal premotor cortex is typically associated with movement-related phenomena (Picard and Strick, 2001). In contrast, the local maximum reflecting imagery-related activity in both mutation and non-mutation carriers (Table 2) is about 16 mm anterior to the primary motor cortex, most likely in the prePMd according to Picard and Strick (Picard and Strick, 2001).

Increases in PMd activity have been observed in non-manifesting Parkin and PINK1 mutation carriers (Buhmann et al., 2005, van Nuenen et al., 2009b), and idiopathic PD patients also show increased PMd activity (Sabatin et al., 2000, Wu and Hallett, 2005). Since different tasks, disease states, and mutation types appear to lead to increased PMd activity, it might be argued that the PMd effect reported in this study is quite un-specific, and that the human motor system deals with the consequences of different mutations by using a single mechanism centered on the dorsal premotor
cortex (van Nuenen et al., 2009b). In fact, close comparison between the current findings, Buhmann et al 2005, and van Nuenen et al 2009 suggests that different portions of the premotor cortex are recruited during performance of motor-related tasks in carriers of recessive mutations (Parkin, PINK1) or dominant mutations (LRRK2). For instance, the internal selection of right thumb movements, studied by Buhmann et al (2005), evoked stronger responses in two frontal clusters of Parkin carriers. One cluster was centered on the mesial frontal cortex (right rCMA, rostral SMA, extending into the adjacent PMd). Another cluster was covering the left PMd, with two local maxima at -39, 6, 42, and -24, -12, 51. Even ignoring the obvious difference in the hemispheric location of the effects found in Buhmann et al (2005) and in the present study, the local maxima of the PMd cluster of Buhmann et al (2005) area 21 and 11 mm apart from the local maximum reported in this study (34, -14,48). The study of van Nuenen et al (2009), comparing the effects of internal selection of finger to thumb movements evoked in Parkin and PINK1 mutation carriers, reports a premotor effect (20, 6, 64) even further away to the effect found in the present study. These data indicate that the premotor effect found in the present study occurs at a different location than those found in Parkin and PINK1 carriers. Taken together with the anatomical differences in striatal impairments observed between carriers of recessive mutations (Parkin, PINK1) and dominant mutations (LRRK2), these findings suggest that different genetic sources of nigrostriatal dopaminergic dysfunction lead to increased activity in different fronto-striatal circuits. However, given that the motor tasks used in these studies (i.e. Buhmann et al, 2005; van Nuenen et al. 2009; present study) also differ in procedures and effectors, it remains to be tested whether different portions of the dorsal premotor cortex, in different hemispheres, would be recruited when carriers with different mutations perform exactly the same task. The increased caudal PMd activity found in LRRK2 carriers might reflect a reduced ability of this region to specify the motor commands required to mentally match the current hand configuration of the subject with the target hand configuration shown on the screen. In this scenario, the increased influence that the right EBA was found to exert on PMd in LRRK2 carriers might be interpreted as a compensatory mechanism for the PMd alteration. EBA is a cortical region originally defined in relation to the visual perception of body parts (Downing et al. 2001). More recent work has also highlighted its involvement in planning voluntary manual actions (Astafiev et al. 2004; Kuhn et al. 2011), namely specifying the goal posture of a planned action (Zimmerman et al. 2011). The increased coupling between EBA and PMd in LRRK2 carriers could reflect increased reliance on visual predictions of the action outcome during the specification of the motor plan evoked by the imagery task. Furthermore, the increased coupling between EBA and PMd in LRRK2 carriers scaled as a function of task demands (hand orientation), after correcting for PMd activity, and with an inverse relation to task-related activity in the caudate. Given the matched behavioral performance between groups, these observations suggest that increased PMd-EBA coupling can be interpreted as compensating the reduced caudate activity observed in LRRK2 carriers. This finding extends and qualifies previous reports on the relevance of compensation for a (latent) nigrostriatal dopaminergic dysfunction during motor execution in symptomatic PD patients (Samuel et al., 1997, Haslinger et al., 2001) and non-manifesting mutation carriers (Buhmann et al., 2005, van Nuenen et al., 2009b).

Conclusion

We have characterized cerebral alterations and potential compensatory mechanisms in asymptomatic LRRK2-G2019S mutation carriers. We show that this mutation leads to movement-related alterations in the caudate nucleus, a feature that distinguishes LRRK2 carriers from the endophenotype of other known genetic subtypes. LRRK2 carriers also show potentially compensatory activity implemented through long-range connectivity between the dorsal premotor cortex and posterior sensory regions, similar to what has been recently observed in idiopathic PD. These findings might capture mechanisms compensating progressive neurodegeneration at the pre-motor stage of PD. Alternatively, these findings might reflect congenital/developmental abnormalities associated with LRRK2, only superficially related to clinical PD by virtue of a common compensatory mechanism deployed across different cerebral phenotypes. A prospective study, following these LRRK2-G2019S mutation carriers, could test the clinical significance of the current findings and their relevance as early markers of PD.

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References


Interference with compensation
Weight-specific anticipatory coding of grip force in human dorsal premotor cortex

BFL van Nuenen, J Kuhtz-Buschbeck, C Schulz, BR Bloem and HR Siebner

Abstract

The dorsal premotor cortex (PMd) uses prior sensory information for motor preparation. Here we used a conditioning-and-map approach in 11 healthy male humans (mean age 27 years) to further clarify the role of PMd in anticipatory motor control. We transiently disrupted neuronal processing in PMd, using either continuous theta burst stimulation (cTBS) at 80% (inhibitory cTBS) or 30% (sham cTBS) of active motor threshold. The conditioning effects of cTBS on preparatory brain activity were assessed with functional MRI, while participants lifted a light or heavy weight in response to a go-cue (S2). An additional pre-cue (S1) correctly predicted the weight in 75% of the trials. Participants were asked to use this prior information to prepare for the lift. In the sham condition, grip force showed a consistent undershoot, if the S1 incorrectly prompted the preparation of a light lift. Likewise, an S1 that falsely announced a heavy weight produced a consistent overshoot in grip force. In trials with incorrect S1, preparatory activity in left PMd during the S1-S2 delay period predicted grip force undershoot but not overshoot. Real cTBS selectively abolished this undershoot in grip force. Further, preparatory S1-S2 activity in left PMd no longer predicted the individual undershoot after real cTBS. Our results provide converging evidence for a causal involvement of PMd in anticipatory down- but not up-scaling of grip force, suggesting an inhibitory role of PMd in anticipatory grip force control during object lifting.

Introduction

Prior sensory information is readily implemented in the preparation and anticipatory guidance of our actions. For instance, people match the applied force to the expected weight of an object when grasping and lifting an object (Johansson and Westling, 1988, Flanagan et al., 2001, Cole and Rotella, 2002). Previous research suggests that the dorsal premotor cortex (PMd) codes predictive aspects of sensory information in the context of manual motor control. The PMd is involved in selecting hand movements based on sensorimotor mapping rules (Picard and Strick, 1996, Passingham et al., 1998, Kurata et al., 2000, Toni et al., 2002, Amiez et al., 2006, van Eimeren et al., 2006). In this process the left PMd plays a dominant role when action selection is based on an arbitrary (non-spatial) mapping rule (Schluter et al., 1998). Specifically, the PMd processes sensory information that is relevant to a pending action and uses it for movement preparation (Boussaoud, 2001, Astafiev et al., 2003, Hoshi and Tanji, 2006, Schubotz, 2007, Grafton et al., 2008).

This presumed role of the PMd has been substantiated by studies in which low-frequency repetitive transcranial magnetic stimulation (rTMS) was used to transiently suppress cortical excitability in the left PMd (Chouinard et al., 2005, Christensen et al., 2007, Nowak et al., 2009). Low-frequency rTMS of the left PMd disrupted the predictive scaling of forces based on arbitrary color cues in a grip-and-lift task (Chouinard et al., 2005, Nowak et al., 2009), and altered the impact of an incorrect predictive cue on subsequent visuomotor mapping (Ward et al., 2010). Motivated by this work, we combined rTMS and functional magnetic resonance imaging (fMRI) to further clarify the functional relevance of the left PMd in implementing prior sensory information into the scaling of grip force. We used continuous theta burst stimulation (cTBS) to mildly and transiently disrupt neural processing in the stimulated left rostral PMd (Huang et al., 2005). In addition, participants underwent fMRI to map the lasting effects of cTBS on preparatory brain activity. During fMRI, subjects performed a grip-and-lift task in which an arbitrary visual pre-cue (S1) correctly (75%) or incorrectly (25%) predicted whether subjects had to lift a heavy or light weight. A second visual cue (S2) which always correctly predicted the object weight triggered subjects to perform the grip-and-lift task. Subjects were asked to prepare for the task based on the information given by the S1 cue. When S1 and S2 cues were incongruent, subjects had to re-adjust the prepared grip according to the S2 information.

The study was designed to test several hypotheses:
(i) At the behavioural level, we reasoned that the prior knowledge about the weight provided by S1 would interfere with optimal anticipatory grip force control, if S1 and S2 were incongruent. We expected a relative overshoot or undershoot of grip force, when subjects wrongly prepared to lift a heavy or light weight, respectively.
We postulated that the regional BOLD signal in PMd should reflect anticipatory coding of predictive information given by the S1 pre-cue (Chouinard et al., 2005, Christensen et al., 2007, Schubotz, 2007, Ward et al., 2010). Specifically, we hypothesized that the level of sustained BOLD activity in PMd during the S1-S2 period should indicate the relative strength of grip force anticipation triggered by the S1 cue and thus, should predict the magnitude of inappropriate grip force scaling in trials were S1 and S2 cues were different.

(iii) Regarding the disruptive effects of cTBS, we predicted that real cTBS would impair the anticipatory force scaling in the stimulated left PMd, resulting in a reduction of relative grip force overshoot and undershoot in trials with different S1 and S2 cues. Likewise, the preparatory activity of the stimulated PMd should no longer predict the grip force behavior after real cTBS of left PMd. Finally, we expected that the disruptive effect of cTBS on anticipatory force control in rostral PMd might be compensated by changes in preparatory activity in the rostral part of the supplementary motor area (SMA), which is also involved in conditional response selection based on arbitrary sensori-motor associations (Kurata et al., 2000).

Materials & Methods

Participants
Eleven healthy male humans (age 27 ± 6.5 years; mean ± SD) without neurological or psychiatric history participated. Subjects were recruited from the student population of the University of Kiel and were naïve to the purpose of the study. Participants were consistently right-handed according to the Edinburgh handedness inventory (Oldfield, 1971). The experimental procedures were approved by the local ethics committee of the Christian Albrechts University. Written informed consent was obtained prior to the study.

Experimental design
We used fMRI to assess the conditioning effects of inhibitory cTBS on regional neural activity during motor preparation (Figure 1). Each participant underwent two experimental sessions in a counterbalanced order. The experimental sessions were identical apart from the cTBS protocol, which used either a biologically real or a very low, sham intensity of stimulation. At least seven days separated the two sessions to exclude carry-over effects of TBS conditioning.

Figure 1A illustrates the order of the experimental procedures. At the beginning of each experimental session, participants were intensively trained on the experimental grip-and-lift task, first outside and then inside the MR scanner, for approximately 20 minutes. The experimental task required participants to grasp and lift a manipulandum with their right dominant hand (for details see below). After participants were familiarized with the motor task, cortical excitability of the left primary motor hand area (M1) was probed with single-pulse TMS of left M1. We then applied either “real” or “sham” cTBS to the left rostral PMd. For “real” TBS (intervention...
condition), stimulus intensity was set at 80% of active motor threshold (AMT), whereas intensity was reduced to 30% of individual AMT during “sham” TBS (control condition). Otherwise, the cTBS protocols were identical. After cTBS conditioning, participants rested for five minutes without moving their hands or feet. We introduced this resting period because previous studies showed that short periods of voluntary motor activity shortly before or after cTBS can modulate the conditioning effects of cTBS on cortical excitability (Huang et al., 2005; Gentner et al., 2008). After the five-minute resting period, single-pulse TMS was again applied to left M1\textsubscript{hand} and MEPs were recorded from right FDI muscle to capture acute TBS-induced changes in corticospinal excitability (post-cTBS, measurement). Participants were then taken to the MR scanner and fMRI started approximately 15 min after the end of cTBS. Participants performed a grasp-and-lift task with their right hand during fMRI. The experimental session was completed by measuring cortical excitability with single-pulse TMS over the left M1\textsubscript{hand} (post-cTBS\textsubscript{2} measurement) in the TMS laboratory. The post-cTBS\textsubscript{2} measurement started approximately 55 min after the end of TBS.

Pre-cued grasp-and-lift task
During fMRI participants carried out a grasp-and-lift task which required precision grips with the right dominant hand (Figure 1B). An MRI-compatible custom-made force transducer (Dasch Instruments, Kiel, Germany) was used for the grasp-and-lift task. The transducer had two flat vertical grip surfaces (40 x 40 mm) spaced 28 mm apart which measured the isometric pinch force exerted between the pads of the thumb and index finger (spring excursion < 0.5 mm) and the load force. The grip surfaces were covered with thin felt. Participants lay supine in the MR scanner with the left arm extended in a comfortable posture. The right arm was extended comfortably so that the right hand rested with a semi-prone posture on a custom-made platform that supported the force transducer. The force transducer was placed between the fingertip of the right thumb and index finger and could therefore be gripped and lifted without any elbow- or shoulder-joint movement. The lateral edges of the force transducer fitted smoothly in grooves of two vertically orientated aluminum bars, so that the device could easily move up and down without tilting. A weight of 100 or 250 g was fixed with a string to the lower end of the transducer. The weights could be changed apart which measured the isometric pinch force. The grip force transducer was placed between the fingertip of the right hand- or shoulder-joint movement. The lateral edges of the force transducer fitted smoothly in grooves of two vertically orientated aluminum bars, so that the device could easily move up and down without tilting. A weight of 100 or 250 g was fixed with a string to the lower end of the transducer. The weights could be changed between trials without the participant being aware of the change in weight. We employed an event-related fMRI paradigm. A single trial lasted on average 12 s and started with the presentation of a symbolic preparatory S1 cue which was presented in the center of a screen 15 cm above subjects’ visual field for 1 s. One of two S1 pre-cues was pseudorandomly presented from trial to trial (Figure 1B). A red circle instructed participants to prepare for grasping and lifting a light weight (100 g), while a red square prompted subjects to prepare for grasping and lifting a heavy weight (250 g) (Figure 1B). An orange cross appeared in the center of the visual field after the S1 cue. Participants were asked to fixate the cross and prepare for the grasp-and-lift task. The cross was presented for 2 to 8 s, and stimulus duration was pseudorandomly varied from trial to trial, resulting in a variable preparatory S1-S2 period. At the end of the preparatory period, a symbolic target cue was centrally presented for 1 s. Again, there were two S2 cues: a green circle instructed participants to grasp and lift the light weight, whereas a green square prompted subjects to grasp and lift the heavy weight (Figure 1B). Participants were asked to lift the manipulandum 2 to 4 cm up and then put the manipulandum back on the platform. After the S2 cue, a grey fixation cross was presented in the centre of the screen for a variable period which was jittered between 2 to 8 s in steps of 1 s.

Participants were instructed to actively prepare for lifting the weight that was indicated by the S1 pre-cue and to grasp and lift the device as fast and as accurately as possible in response to the S2 target cue. In 75% of the trials the preparatory S1-cue correctly predicted the S2 cue. Participants were explicitly informed about the predictive value of the S1 cue. Participants were informed that S1 would correctly predict the forthcoming weight in the majority of trials, and were instructed to lift the weight swiftly and as accurately as possible also in incorrectly pre-cued trials. The experimental task resulted in four experimental conditions of interest. There were two conditions in which the S1 pre-cue and S2 target cue had the same shape. In these trials, the S1 pre-cue correctly predicted the weight that had to be lifted. Participants prepared for a heavy lift and then lifted the heavy weight (referred to as “HH” condition) or they prepared for a heavy lift and lifted the light weight (referred to as “LL” condition). In the remaining two conditions, the S1 and S2 pre-cue differed in shape and thus, the S1 cue was incorrect. Here the S1 pre-cue either triggered the preparation for a light lift, but the S2 target cue indicated a heavy lift (referred to as “HL” condition) or the S1 pre-cue indicated a heavy lift, but subjects were then instructed to lift the light weight (referred to as “LH” condition). There was also a fifth experimental condition which served as low-level control condition. In these “control trials”, a blue diamond was presented as S1 and S2 cue in the center of the screen (Figure 1B). These cues were of no behavioral relevance. Participants had only to lay still and watch these cues without preparing for or performing any grip or lift.

Functional magnetic resonance imaging
The fMRI measurements were split in two consecutive runs. Each fMRI run included 40 grasp-and-lift trials (10 trials per experimental condition) and 15 control trials which were intermingled in a pseudorandom order. A single run lasted for 11 min.
There was always an examiner (BvN or CK) in the MR-room who changed the weights (100 and 250 g) from trial to trial. A cue which was only visible to the examiner indicated which weight was to be lifted in the next trial.

MRI was performed on a 3.0 T Philips Achieva MR scanner with an eight-channel array head coil (Philips, Best, The Netherlands). Participants wore headphones for noise protection, and foam pads restricted head motion. We used a T2*-weighted gradient echo planar imaging (EPI) sequence with an echo time of 35 ms. The field of view covered the whole brain (230 x 230 mm) and a pixel size 2.9 x 2.9 mm. Each EPI volume was obtained within 3000 ms (repetition time) and comprised 36 axial slices with a voxel size of 2.9 x 2.9 x 3.0 mm and interslice gaps of 0.3 mm. We also obtained a whole-brain structural MRI dataset using a three-dimensional T1-weighted FLASH sequence (repetition time 7.7 ms, axial field of view: 230 mm, 160 contiguous slices, voxel size 1 x 1 x 1 mm).

**Measurement of corticospinal excitability with TMS of left M1**

Motor cortical excitability was assessed with single-pulse TMS over the left M1\_HAND using a biphasic pulse configuration and a figure-of-eight shaped ‘MC-B70’ coil with an outer diameter of 70 mm connected to a MagPro-100 stimulator (MagVenture, Farum, Denmark). TMS was applied while participants were comfortably seated in an armchair with the head stabilized by a neck rest. Both arms were supported by a cushion to facilitate complete relaxation of the arm and hand muscles. Subjects were instructed to relax but to keep their eyes open and fixate a wall two meters in front of them.

The coil was positioned tangentially to the skull over the left M1\_HAND with the handle pointing backwards and laterally at an angle of approximately 45° to the sagittal plane. At this coil orientation, the second phase of the biphasic TMS pulse induces an electrical current in the brain tissue with a posterior-lateral to anterior-medial direction roughly perpendicular to the central sulcus which is optimal for evoking a motor response in the contralateral hand (Mills et al., 1992).

We defined the scalp site where a single TMS pulse at slightly suprathreshold intensity consistently yielded maximal Motor Evoked Potential (MEP) in the right contralateral first dorsal interosseus (FDI) muscle. This “motor hot spot” was used as stimulation site for all TMS measurements and used as anchor point to define the site for TBS of the left PMd. To individually adjust the stimulus intensity, we determined the resting and active MT. We first determined the resting MT in the relaxed FDI muscle which was defined as the minimum stimulus intensity that produced an MEP of more than 50 µV in five out of 10 consecutive trials. We then measured the active MT defined as the lowest stimulus intensity at which MEPs were elicited in five out of 10 consecutive trials during tonic contraction of the FDI muscle at about 10% of maximum force level using a criterion for the MEP of 100–250 µV peak-to-peak amplitude. MTs were determined by gradually decreasing and increasing the stimulus intensity in steps of 1% of maximum stimulator output. MEPs were recorded with surface electromyography (EMG). Ag–AgCl disc surface electrodes were attached over the right FDI muscle using a belly-tendon montage. Changes in corticospinal excitability were assessed over the left M1\_HAND with single-pulse TMS using a stimulus intensity which elicited MEPs with approximately 1 mV peak-to-peak amplitude in the right FDI muscle. The stimulus intensity was determined at baseline in the real and sham TBS sessions and then kept constant across the entire experimental session. The reference electrode was placed at the wrist. EMG activity was continuously monitored using visual (oscilloscope) and auditory (speakers) feedback to ensure complete relaxation at rest and a constant level of EMG activity during tonic contraction. The raw EMG signals were amplified by 1000 (D360, Digitimer Ltd, Welwyn Garden City, Herts, UK), filtered between 20 and 1000 Hz, and digitized at 5000 Hz per channel (CED Power1401, 16-bit-ADC; Cambridge Electronic Design, Cambridge, UK). The administration of TMS pulses as well as EMG data recording, storage, and analyses was performed with Signal software (Cambridge Electronic Design, Cambridge, UK).

**Continuous theta burst stimulation of left dorsal premotor cortex**

We used cTBS for conditioning of left PMd because cTBS produces a lasting suppression of regional excitability in the stimulated cortex (Huang et al., 2005). The cTBS protocol involved repeated administration of short high-frequency bursts. Each burst consisted of three pulses given at an inter-stimulus interval (ISI) of 20 ms (corresponding to a rate of 50 Hz). These high-frequency triple-pulse bursts were repeated every 200 ms. Theta burst stimulation was given to left PMd as a continuous train lasting for 40 s. The site for PMd stimulation was defined in relation to the “motor hot spot” with the coil being placed 2 cm anterior and 1 cm medial to the left M1\_HAND. This coil positioning procedure used the functionally localized M1\_HAND as anchor point and was adopted from Schluter et al. (Schluter et al., 1998) who used this coil positioning procedure to interfere with processing in left PMd.

The intensity of real cTBS was set at 80% of the individual AMT \( \text{cTBS}_{\text{real}} \) for sham cTBS we used an intensity of 30% of the individual AMT \( \text{cTBS}_{\text{sham}} \). The latter intensity was predicted to be ineffective in terms of inducing action potentials in the PMd. We opted for low-intensity cTBS rather than using a sham coil because we wished to induce somatosensory stimulation of the scalp during sham cTBS (Helmich et al., 2006).
Data analysis

Analysis of grip and lift force data

The non-metallic custom-made force transducer (Dasch Instruments, Kiel, Germany) was connected to the computer based SC/ZOOM data acquisition and analysis system (Department of Physiology, Umeå University, Sweden) via a fiber optic connector. Grip and lift forces were sampled at a rate of 200 Hz. We focused our analysis on the initial changes in grip- and load force until the first peak to assess initial preparatory scaling of grip force (Figure 3). Four force measures were determined for each trial: (i) peak grip force (GF), (ii) peak load force (LF), (iii) peak rate of grip force (GFR), and (iv) peak rate of load force (LFR). We also calculated the reaction time (RT), which was defined as the time between the onset of the S2 target cue and the onset of increase in GF. Mean values of each measure were calculated for each of the four experimental conditions of interest (i.e., HH, LL, LH and LH).

In a first step, we explored the patterns of normal task performance without perturbation of left PMd using only the data recorded after sham TBS. We computed separate two-factorial repeated measures of analyses of variance (ANOVA) with the factor weight (2 levels: 100 g vs. 250 g) and S1 validity (2 levels: correct vs. incorrect S1 precue) using GF, LF, GFR, LFR, and RT as dependent variable. We also performed an additional three-factorial ANOVA which included the factor type of intervention (2 levels: real cTBS vs. sham cTBS) to assess the conditioning effects of real cTBS on grip force control. We were particularly interested in the behavioural consequences of an incorrect pre-cue on grasping and lifting. To this end, we calculated the ratio between the GF in incorrectly pre-cued and correctly pre-cued trials for each weight (GF/ GFcorrect) and GF/ GFincorrect. The same ratio was also calculated for the other grip and lift force measures (i.e., GFR, LF and LFR). Using these ratios as dependent variables, two-factorial ANOVAs tested whether the absolute weight (2 levels: 100 g vs. 250 g) or the type of intervention (2 levels: real cTBS vs. sham cTBS) influenced the effect of the incorrect pre-cue on task performance.

Previous grip force studies have consistently shown that the somatosensory Information acquired by a recent lift influences the predictive scaling of forces for a subsequent lift (Johansson and Westling, 1988, Gordon et al., 1993, Chouinard et al., 2005). Therefore, we performed supplementary two-factorial ANOVAs with the factor weight (heavy vs. light weights) and compatibility with previous lift (same weight vs. different weight) for the real cTBS and sham cTBS session.

Prompted by the reviewers comment, we performed an additional analysis which assessed the impact of the last trial on grip force control. In agreement with previous work, applied grip force was influenced by the weight of the previous trial. When the previous trial required a heavy lift, subjects showed a relative overshoot in peak grip force when lifting a light weight. Conversely, there was a relative undershoot in peak grip force, when a heavy lift followed a light lift. Critically, this effect was not influenced by the type of TMS being significant after sham cTBS. F(10)=38.49; p<0.001 and after real cTBS F(10)=22.19; p=0.001. Greenhouse-Geisser (GG) correction for non-sphericity was applied if necessary. Conditional on significant F-values, ANOVAS were followed by post-hoc two-sided paired-sample t-tests. Statistical threshold was set at P ≤ 0.05. Group data is given as mean ± standard deviation if not specified otherwise.

Analysis of motor evoked potentials

Peak-to-peak amplitudes (mv) of the MEP recorded from the right FDI muscle were measured trial-by-trial and mean MEP amplitudes were calculated for each block of measurements (NuCursor software, Sobell Department of Motor Neuroscience and Movement Disorders, Institute of Neurology, Queen Square, London, UK). Repeated measures ANOVAs were used to test for lasting effects of cTBS over left PMd on excitability of ipsilateral left M1. The ANOVA model included the factors type of intervention (2 levels: real cTBS vs. sham cTBS) and block of measurement (3 levels: baseline, measurements starting 5 and 55 min after TBS conditioning). ANOVAs were followed by post-hoc two-sided paired-sample t-tests conditional of significant F-values. For all analyses a significance level of P < 0.05 was applied after non-sphericity (GG) correction.

Analysis of the fMRI data

The fMRI data were processed and analyzed using statistical parametric mapping (SPM) software (Wellcome Trust Centre for Neuroimaging, London, UK; http://www.fil.ion.ucl.ac.uk/spm). The first two scans of each session were discarded to allow for steady-state magnetization. The remaining images were realigned to the first image and spatially normalized to MNI stereotactic space using a standard EPI template as implemented in SPM. The normalized images were spatially smoothed with a Gaussian kernel of 9 mm full-width at half-maximum. At the individual level, we constructed a general linear model which comprised both experimental sessions and took into account the factorial design. The presentation of the S1 onset pre-cue, the variable interval between S1 and S2 (i.e. preparatory period), and the onset of the S2 pre-cue were modeled separately for each of the five trial types (i.e., HH, LL, LH, HL, and control trials) using delta functions convolved with a hemodynamic response function (HRF). Based on this model we computed t-statistical maps which expressed regional changes in BOLD signal for experimental contrasts of interest for each voxel in the brain.
Second level analysis tested for experimental modulations of the regional BOLD signal during the preparatory S1-S2 period. The data for the second stage of analysis comprised pooled parameter estimates for each contrast of interest across all subjects in a random effects analysis. Contrast images for each subject were entered into a one sample t-test for each contrast of interest to identify brain regions which increased their neuronal activity (as indexed by the BOLD signal) in the preparatory period between the S1 precue and the S2 target cue. Another t-test was computed to identify brain regions where the real cTBS\textsubscript{pm} protocol increased or decreased regional preparatory activity relative to the sham TBS\textsubscript{pm} condition. We also used a one-sample t-test for main-effects of brain activity during the different conditions. A paired t-test was calculated to test for weight specific brain activities during the preparatory phase for preparing to lift a heavy or light weight. The mean difference in grip force between the correctly and incorrectly pre-cued trials for each weight were included in the analysis as covariate of interest to test whether inter-individual variations in preparatory brain activity during the S1-S2 period correlated with inter-individual differences in the behavioural impact of the incorrect pre-cue on task performance.

In addition to rostral PMd, it has been shown that the rostral part of the SMA is also involved in conditional response selection based on arbitrary sensori-motor associations.(Sakai et al., 1999, Kurata et al., 2000) This raises the possibility that activity in rostral SMA might compensate for the disruptive effects of real cTBS\textsubscript{pm} on anticipatory force control in the stimulated left rostral PMd. Therefore, we used simple regression analysis to test whether the effects of real cTBS\textsubscript{pm} on anticipatory grip force control were less pronounced in subjects in whom real cTBS\textsubscript{pm} Changed preparatory S1-S2 activity in rostral SMA.

All t-tests carried out within SPM were one tailed and included all voxels within the brain. The height threshold for the resulting statistical parametric maps (t-score maps) was set at an uncorrected p-value of p < 0.01. All SPMs were transformed to the unit normal Z-distribution to create a statistical parametric map (SPM). P-values were corrected at the cluster level applying an uncorrected extent threshold of P < 0.01. A cluster that failed to meet the significance criterion but consisted of more than 50 contiguous voxels is reported as statistical trend if the peak voxel in the cluster exceeded an uncorrected P < 0.001.

Since cTBS targeted the left rostral PMd and our experiment was specifically designed to explore the role of left PMd in the implementation of advance sensory information in movement preparation, the left rostral PMd was defined as \textit{a priori} region of interest (ROI). The rostral SMA was defined as second ROI since we expected the rostral SMA to compensate for the lesion effect induced by real cTBS\textsubscript{pm} of left PMd.

Within the two ROIs statistical threshold was set to p < 0.001 (uncorrected). The spherical ROIs (14 mm radius) were placed into the left PMd and left rostral PMd, centered on MNI stereotactic coordinates (x = -24, y = -3, z = 54 for PMd and x = -9, y = 9, z = 51 for SMA) that correspond to published activation peaks during a visuospatial response selection task (van Eimeren et al., 2006).

For left rostral PMd and rostral SMA, correction for multiple comparison was only performed for all voxels within the ROI. For all remaining voxels, statistical results were corrected across the whole brain.

All statistical parametric maps are superimposed onto a T2-weighted structural MRI template provided by MRicro (http://www.cabiatl.com/mricro/micro/index.html). The voxels of the activation maps are color-coded according to their Z values and for illustrative purposes thresholded at an uncorrected p value of p < 0.01.

**Results**

**Changes in corticospinal excitability in left M1\textsubscript{HAND}**

Real cTBS\textsubscript{pm} induced a sustained decrease in mean MEP amplitude in the right FDI muscle, which was not found after sham cTBS\textsubscript{pm} (Figure 2). A differential effect of the two rTMS protocols on corticospinal excitability in ipsilateral M1\textsubscript{HAND} was confirmed by the ANOVA, showing an interaction between type of intervention and block of measurement (F(2,20) = 9.62, p = 0.001). Post-hoc paired t-test revealed significant decrease of the MEP amplitude 5 min after the end of real cTBS\textsubscript{pm} (t(10) = 5.123, p < 0.001) and 55 min after the end of real cTBS\textsubscript{pm} (t(10) = 4.64, p = 0.001), but no consistent changes after sham cTBS\textsubscript{pm}.

There were no differences in resting MT, active MT or mean MEP amplitude at baseline between the two experimental sessions. This lasting decrease in corticospinal excitability in ipsilateral M1\textsubscript{HAND} was comparable to the inhibitory after effects that have been reported in previous 1Hz rTMS studies(Gerschlager et al., 2001, Chouinard et al., 2003, Suppa et al., 2008). This inhibitory effect of real cTBS\textsubscript{pm} in M1\textsubscript{HAND} excitability did not correlate with the cTBS-induced changes in grip force undershoot, nor did it correlate with cTBS-induced changes in weight-specific preparatory activity. This may be due to the fact that corticospinal excitability was assessed at rest, whereas the behavioral and fMRI measures were obtained during an active motor context during a pre-cued grip-and-lift task.

**Grip force control**

All participants found the tasks easy to perform. Error rate was very low with less than 2 error trials per fMRI session and are not considered further. The validity of the pre-cue had consistent effects on task performance. Compared to correct
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Pre-cues, incorrect pre-cues resulted in longer mean reaction times ($F(1,10)=14.9; p=0.003$). Neither the absolute weight that had to be lifted nor the intensity of cTBS influenced mean RT. There were no significant interactions among the experimental factors that influenced mean RT.

Table 1 summarizes the group data for the grip force measures of interest. The weight that had to be lifted in a given trial had a consistent effect on all four force measures with higher force and force rate levels when subjects grasped and lifted the heavy weight (GF, GFR, LF, LFR; $p < 0.001$). The measurements during the control session in which we applied sham TBS at very low intensity revealed that the validity of the S1 pre-cue affected the force generation. Incorrect pre-cues produced opposite effects on force measures during heavy or light lifts: gripping and lifting a light weight was associated with a higher GF, GFR and LFR in incorrectly pre-cued trials (HL) than correctly pre-cued (LL) grip-and-lift trials (Figure 3; right).

![Figure 2](image-url)

Figure 2: Conditioning effects of cTBS to left PMd on corticospinal excitability.

Group data of relative changes in mean peak-to-peak amplitude of the motor evoked potentials (MEPs) normalized to the mean amplitudes after real cTBS of left PMd. The open squares represent the MEP amplitudes after sham TBS of left PMd. The open diamonds represent the MEP amplitudes after sham TBS of right PMd. The filled squares give the MEP amplitudes after real cTBS of left PMd. The filled diamonds represent the MEP amplitudes after real cTBS of right PMd. The first post-cTBS measurement was performed 5 minutes after the end of cTBS prior to fMRI. The second post-cTBS measurement was carried out after the end of the fMRI session (i.e., approximately 55 min after the end of cTBS). cTBS = continuous theta burst stimulation. AMT = active motor threshold.

Table 1: Group values for each variable

<table>
<thead>
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<th></th>
<th>LL</th>
<th>HL</th>
<th>HH</th>
<th>LH</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT (sec)</td>
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<td>0.57 (0.19)</td>
<td>0.53 (0.15)</td>
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<td>11.25 (4.56)</td>
<td>10.86 (4.35)</td>
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<tr>
<td>GF rate (N/s)</td>
<td>39.51 (30.34)</td>
<td>45.89 (31.06)</td>
<td>69.24 (28.73)</td>
<td>63.76 (34.04)</td>
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<tr>
<td>LF (N)</td>
<td>2.11 (1.3)</td>
<td>2.15 (1.21)</td>
<td>5.22 (1.74)</td>
<td>5.15 (1.58)</td>
</tr>
<tr>
<td>LF rate (N/s)</td>
<td>25.16 (17.43)</td>
<td>26.17 (15.91)</td>
<td>44.98 (22.79)</td>
<td>41.76 (18.51)</td>
</tr>
</tbody>
</table>

Group values of each behavioural variable for the four experimental conditions, separately for the two experimental conditions. RT = reaction time; GF = Grip force; LF = Load force; N = Newton; s = second; LL = correctly cued light lift; HL = incorrectly cued light lift; HH = correctly cued heavy lift; LH = incorrectly cued heavy lift; SD = standard deviation.
The same pattern emerged when comparing the ratios between incorrectly and correctly pre-cued trials (i.e., comparing the HL:LL ratio and LH:HH ratio). Paired-t-tests revealed highly significant differences between the two ratios for GF\textsubscript{sham}, GFR\textsubscript{sham} and LFR\textsubscript{sham} (p < 0.001). Individual HL:LL ratios were mainly above 1 reflecting the overshoot triggered by the incorrect “HEAVY” pre-cue. Conversely, individual LH:HH ratios were consistently below 1 reflecting the undershoot induced by the incorrect “LIGHT” pre-cue. With respect to the relative magnitude, the undershoot as indexed by the LH:HH ratio (Figure 4; black bars) was less pronounced compared to the overshoot as indexed by the HL:LL ratio (see figure 4; white bars). Paired-t-tests revealed highly significant differences between the two ratios for GF\textsubscript{sham}, GFR\textsubscript{sham} and LFR\textsubscript{sham} (p < 0.001).

**Figure 3** Impact of the validity of the pre-cue on the grip force curves. Mean gripforce (GF), gripforce rates (GF rate), loadforce (LF) and loadforce rates (LF rate) for the four different trial types of a representative subject. Normal lines indicate the correctly precued trials; the dotted lines represent the mean data of the incorrectly precued trials. The left panel depicts the mean data of trials requiring subjects to lift a heavy weight, while the right panel shows the mean data of trials requiring a light lift. Trial types according to S1-S2 sequence: HH = heavy-heavy, LL = light-light, LH = light-heavy, HL = heavy-light.

**Figure 4** Effect of the validity of the pre-cue on grip and lift force. The white columns give the mean overshoot in force production caused by an incorrect S1 pre-cue indicating a heavy weight. The overshoot corresponds to the ratio between HL and HH trials. The black columns give the mean undershoot in force production caused by an incorrect S1 pre-cue indicating a light weight. The undershoot corresponds to the ratio between LH and LL trials. Trial types according to S1-S2 sequence: HH = heavy-heavy, LL = light-light, LH = light-heavy, HL = heavy-light). Error bars indicate standard deviation. GF = grip force; LF = lift force. * = paired t-test p < 0.001.
We tested whether the magnitude of the effect of S1-S2 discrepancy, as reported in Fig. 4, on force responses is entirely accountable for by S1, or whether the S1-S2 discrepancy contributes to the execution of the force response planned by the same S1 weight cue. Paired-t-tests revealed highly significant differences (p < 0.001) by directly comparing the gripforces (GF, GFR, LF and LFR) form HL vs HH and LH vs LL, indicating that the force response was not entirely planned based on the information provided by S1. The data rather indicate that the predictive information provided by S1 interfered with the information provided by S2, causing a response conflict.

A two-factorial repeated-measures ANOVA with the factor weight (heavy vs. light weight) and compatibility with previous lift (same weight vs. different weight) was performed to test whether the weight of the previous lift influenced predictive grip force control. In agreement with previous work (Johansson and Westling, 1988, Gordon et al., 1993, Chouinard et al., 2005), the ANOVA showed that grip force was influenced by the weight of the previous trial. When the previous trial required a heavy lift, subjects showed a relative overshoot in grip force when lifting a light weight. Conversely, subjects showed a relative undershoot in grip force when a heavy lift followed a light lift.

Critically, this effect was not influenced by the type of TMS being significant after sham cTBS: F(10)=38.49; p<0.001 as well as after real cTBS F(10)=22.19; p=0.001). Post-hoc paired T-tests revealed for the sham cTBS session a significant decrease in GF for heavy weights when the previous lift was a light weight (mean±SD 11.89±4.0 N for previous heavy weight and 11.28±3.9 N for previous light weight; t(10)=3.49; p=0.006) and a significant increase in GF for light weights when the previous lift was a heavy weight (mean±SD 5.39±3.0 N for previous light weight and 6.0±3.0 N for previous heavy weight; t(10)=5.35; p<0.006). Post-hoc paired T-tests revealed for the real cTBS session a significant decrease in GF for heavy weights when the previous lift was a light weight (mean±SD 11.62±3.5 N for previous heavy weight and 10.82±3.5 N for previous light weight; t(10)=3.38; p=0.007) and a significant increase in GF for light weights when the previous lift was a heavy weight (mean±SD 4.73±2.0 N for previous light weight and 5.8±2.6 N for previous heavy weight; t(10)=3.62; p<0.005).

**Effect of premotor cTBS on anticipatory grip force control**

We also tested whether real cTBS changed the pattern of task performance as opposed to performance after sham cTBS at 30% of AMT. A two-factorial repeated-measures ANOVA including the factors force ratio (2 levels; HL/LL and LH/HH) and type of intervention (2 levels, cTBS and sham cTBS) revealed a significant interaction between ratio and intervention only for GF (F(1,10) = 4.79; p = 0.05), but not for the other three variables.

Figure 5 plots the relative overshoot (HL:LL ratio) and undershoot (LH:HH ratio) of mean GF separately for the sessions in which real cTBS or sham cTBS was given to left PMd. While the relative overshoot was comparable between the two experimental sessions, there was a difference in the relative undershoot. Real cTBS of left PMd abolished the relative undershoot in LH trials when the pre-cue had incorrectly announced a light weight (t(10)=2.51, p = 0.031). In contrast, the relative overshoot in HL trials was not changed by real cTBS when an incorrect pre-cue had wrongly announced a heavy weight (p > 0.3). The individual changes in the magnitude of undershoot in LH trials did not correlate with the individual changes in corticospinal excitability after real cTBS (r=-0.12; p=0.72).

Figure 5: Effect of real cTBS of left PMd on maximal grip force. The white columns give the mean overshoot in force production (i.e., the ratio between HL and HH trials) caused by an incorrect S1 pre-cue indicating a heavy weight. The black columns give the mean undershoot in force production (i.e., the ratio between LH and LL trials) caused by an incorrect S1 pre-cue indicating a light weight. Compared to sham cTBS, real cTBS abolished the undershoot in maximal grip force caused by an incorrect "light-weight" cue. The asterisk denotes a significant difference of the pair-wise comparison at p <0.05. Error bars indicate standard deviation.
Distinct clusters in left and right PMd, left supramarginal gyrus, as well as left and right medial intraparietal sulcus (IPS) showed significant greater activation when participants prepared for lifting the heavy weight as opposed to preparing for lifting the light weight (Figure 6B). The relative increase in preparatory activity for the heavy weight was significant in the left PMd (peak at x, y, z = -27, -15, 60; Z = 3.04, P_{SVC} = 0.001). No cluster in the brain showed increased preparatory activity for the light-weight relative to the heavy-weight cue, even when applying a liberal threshold of p<0.01 (uncorrected).

Preparatory activity in left PMd predicts undershoot in grip force
We hypothesized that in the absence of real cTBS, the level of preparatory activity in the left PMd would predict the behavioural impact of the incorrect S1 pre-cue on force generation. To test this hypothesis, we performed a second-level regression analysis based on the preparatory activity during the control fMRI session (i.e., after sham cTBS). In each subject, we calculated the mean LH:HH ratio of peak grip force during the control session which indicates the relative undershoot of grip force when lifting the heavy weight after having prepared to lift the light weight. Using the LH:HH ratio as covariate of interest, we found that the magnitude of preparatory activity (of light weights) in left rostral PMd correlated with the relative undershoot in maximal grip force in the LH condition (peak correlation at x, y, z = -30, -3, 54; Z = 3.73, P_{SVC} = 0.025). The greater the preparatory activity in left PMd, the greater was the relative undershoot in peak grip force when lifting the heavy weight after preparing to lift the light weight (Figure 7A).

We performed the same type of regression analysis using the individual HL:LL ratio of peak grip force. The HL:LL ratio reflects the relative overshoot in grip force when lifting the light weight after having prepared to lift the light weight. Using the LH:HH ratio as covariate of interest, we found that the magnitude of preparatory activity (of light weights) in left rostral PMd correlated with the relative undershoot in maximal grip force in the LH condition (peak correlation at x, y, z = -30, -3, 54; Z = 3.73, P = 0.025). The greater the preparatory activity in left PMd, the greater was the relative undershoot in peak grip force when lifting the heavy weight after preparing to lift the light weight (Figure 7A).

Preparatory brain activity after inhibitory theta burst stimulation of left PMd
The level of preparatory neuronal activity in left PMd was not altered by the real cTBS protocol compared to control cTBS. However, real cTBS of left PMd abolished the relationship between the preparatory activity in the stimulated left PMd and the amount of undershoot in grip force.
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PMd and the behavioural effect of incorrect pre-cueing on force generation (Figure 7B). In the control session with low-intensity cTBS (30%) of left PMd, the preparatory activity of an incorrect “LIGHT” pre-cue on the undershoot in grip force (r = 0.85; P = 0.001), whereas preparatory activity in the stimulated left rostral PMd no longer predicted individual variations in the relative undershoot in LH trials after real cTBS (80%) (r = 0.24; P = 0.48).

We examined whether inter-individual variations in the effects of real cTBS on anticipatory grip force control (i.e., the reduction in grip force undershoot in LH trials) were associated with inter-individual variations in cTBS-induced changes in preparatory S1-S2 activity. Specifically, we were interested to test whether the inter-individual differences in cTBS-induced change of GF undershoot (i.e., preparatory S1-S2 activity for light lifts relative to heavy lifts) were correlated with cTBS-induced changes in weight-specific preparatory activation.

Confirming our hypothesis, a cluster in left rostral SMA (peak correlation at x, y, z = -6, 18, 54; Z = 3.32; P < 0.001 and x, y, z = -15, 12, 57; Z = 2.99; P = 0.001) showed a linear relationship between the cTBS effect on weight-specific preparatory activity and undershoot in LH trials (r = 0.868; p = 0.001; Figure 8): In subjects showing a relative increase in preparatory S1-S2 activity for light lifts (relative to heavy lifts) after real cTBS, real cTBS did not affect the undershoot in response to an incorrect S1 pre-cue (Figure 8). Conversely, real cTBS induced a clear reduction in grip force undershoot in those subjects showing no increase in preparatory S1-S2 activity for light lifts (relative to heavy lifts) (Figure 8). Additional clusters showing the same linear relationship were located in the left globus pallidus internus (peak at stereotactic coordinates x, y, z = -12, 3, 6; Z = 3.78; P < 0.001) and right medial prefrontal cortex (peak at x, y, z = 9, 57, 33; Z = 3.95; P < 0.001).

We also tested for a linear relationship between the individual decreases in MEP amplitude after real cTBS and preparatory activity during the task. The inter-individual variation in MEP suppression did not correlate with individual changes in BOLD signal in the left rostral PMd during the preparatory period (r = 0.038; P = 0.917).
Discussion

The experiments yielded three main findings. First, cTBS\textsubscript{80\%} of left PMd selectively impairs anticipatory down-scaling, abolishing the grip force undershoot but not overshoot in trials with incorrect S1. Second, in the absence of cTBS\textsubscript{80\%}, individual variations in preparatory activity of left PMd as triggered by a “light weight” cue predicted the relative grip force undershoot in LH trials. Third, this association between preparatory activity in left PMd and individual variations in grip force undershoot was cancelled by cTBS\textsubscript{80\%} of left PMd. Taken together, these experiments offer the first demonstration that human left PMd contributes to anticipatory down-scaling of grip force based on arbitrary visual cues.

Anticipatory grip force control based on arbitrary visual cues

Grip-and-lift tasks involving small objects have been used intensively to study the role of prediction on sensorimotor control (Flanagan et al., 2006, Johansson and Flanagan, 2009). Here, we used a novel S1-S2 paradigm in which predictive grip force control was informed by prior visual information based on arbitrary cues. In contrast to previous work (Chouinard et al., 2005), anticipatory force scaling was challenged by introducing a conflict between two “predictive” visual cues, an incorrect S1 pre-cue and a correct S2 go-cue, rather than by causing a conflict between an incorrect visual cue and somatosensory feedback during the task signaling the prediction error. Because the S2 go-cue was always correct, task performance always created somatosensory feedback that was concordant with the predictive visual information provided by this cue. However, including a S1 cue did not prevent the subjects to take the previous lift into account for the scaling of grip force, therefore we propose that there are two scaling mechanisms which are reflected in the behavioural data (proprioceptive and visual), but cTBS\textsubscript{80\%} only influenced the visually cued interference and not the proprioceptive interference. These results are in line with previous data showing that the proprioceptive information gained during the previous can be disturbed by inhibiting the primary cortex and that the visuomotor information is stored in the PMd (Chouinard et al., 2005).

Although the S2 go-cue always provided the correct information about object weight, the incorrect S1 cue still interfered with anticipatory force control causing an undershoot (in case of an incorrect “light weight” pre-cue) or an overshoot (in case of an incorrect “heavy weight” pre-cue). This finding indicates that subjects actually used the S1 pre-cue for motor preparation. It further shows that the correct predictive information provided by the S2 go-cue was not sufficient to rapidly discard the inappropriate preparatory set evoked by the incorrect S1 stimulus. The longer reaction times after an incorrect S1 stimulus indicates that grip initiation was delayed in order to allow for partial reprogramming of grip force (Loh et al.,...
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Without a delay in reaction time, the undershoot (after an incorrect “light weight” pre-cue) and the overshoot (after an incorrect “heavy weight” pre-cue) might have been even higher. Interestingly, the relative overshoot caused by an incorrect “heavy weight” pre-cue in HL-trials was more pronounced in magnitude as opposed to the relative undershoot produced by incorrect “light weight” pre-cues in LH-trials. This might reflect a general bias of the motor system to apply too much rather than too little grip force in order to avoid dropping the object.

The variable delay between the S1 pre-cue and the S2 go-cue enabled us to dissociate preparatory activity in left PMd from event-related activity evoked by the visual cues, or by task performance itself. We reasoned that if left PMd codes the predictive information revealed by the S1 pre-cue, preparatory activity in left PMd should predict the behavioral consequences of an incorrect S1 pre-cue on force scaling. In fact, preparatory activity of left PMd predicted inter-individual variations in grip force undershoot following an incorrect “light weight” pre-cue. This was, however, not the case when an incorrect “heavy weight” pre-cue caused an overshoot in force scaling. Here it was the SMA rather than the PMd showing a correlation between preparatory activity and inter-individual variations in grip force undershoot in HL-trials. The results suggest that the left PMd is primarily concerned with predictive down-scaling of grip force, while other premotor areas such as the SMA might preferentially support predictive up-scaling of grip force in humans (Vaillancourt et al., 2007, Haller et al., 2009).

Causal involvement of PMd in anticipatory force scaling

A recent electrophysiological study in two monkeys supports the notion that inhibitory processes in PMd might prevail during motor preparation (Kaufman et al., 2010). Extracellular recordings were obtained from chronically implanted multi-electrode arrays in contralateral PMd while monkeys performed a visually guided reaching task. Recordings revealed a significant rise in overall firing rate of inhibitory interneurons, but not pyramidal cells during the delay period. This raises the possibility that in our fMRI measurements, the BOLD signal increase in left PMd abolished predictive grip force undershoot in trials with incorrect S1-S2 period following a “light-weight” pre-cue, whereas preparatory activity coding the anticipated force dominated the S1-S2 period following a “heavy-weight” pre-cue. This would explain why S1-S2 activity in PMd only predicted the relative force undershoot in LH-trials with an incorrect “light-weight” pre-cue, but not overshoot in HL-trials with an incorrect “heavy-weight” pre-cue.

We propose that one important role of the PMd is to prevent excessive motor activity during motor preparation and execution. When preparing for a heavy lift, PMd activity during the S1-S2 period is more concerned with preventing a premature grip. Conversely, the PMd is more engaged in preparatory down-scaling of the grip force level when preparing for a light lift. A similar effect, albeit not reaching significance, was also found for lift force control in the present study. Furthermore, several previous neuroimaging studies reported higher premotor activity, the less force had to be applied across a range of manual pre-cues, anticipatory grip force control had to integrate the diverging visual information provided by the S1 and S2 cue. Our task enabled us to assess the specific involvement of PMd in anticipatory up- and down-scaling of grip force based on arbitrary visual cues. Extending previous work (Chouinard et al., 2005, Nowak et al., 2009), we show that a transient disruption of the PMd selectively impaired down-scaling of force if a visual mode of anticipatory control is reinforced by the paradigm.

The preparatory S1-S2 period did not only require to prepare for the grip and lift, but also to withhold the grip until the appearance of the S2 go-cue. Application of the GABA antagonist bicuculline in monkeys to the PMd reduces the ability to withhold reaching movements of the contralateral limb in a visually guided reaching task (Sawaguchi et al., 1996). This raises the possibility that in the present study, regional PMd activity during the S1-S2 period was not only related to motor preparation but also to preventing a premature grip. This inhibitory activity should be larger in trials in which subjects prepared for a heavy lift. In fact, we found a higher level of S1-S2 activity in left PMd when subjects prepared for lifting a heavy weight as opposed to a light weight. We hypothesize that in left PMd, inhibitory activity preventing a premature motor response prevailed in the S1-S2 period following a “heavy-weight” pre-cue, whereas preparatory activity coding the anticipated force dominated the S1-S2 period following a “light-weight” pre-cue. This would explain why S1-S2 activity in PMd only predicted the relative force undershoot in LH-trials with an incorrect “light-weight” pre-cue, but not overshoot in HL-trials with an incorrect “heavy-weight” pre-cue.
tasks, including a power grip task (Ward and Frackowiak, 2003), index finger abduction (van Duinen et al., 2008), static precision grip (Kuhtz-Buschbeck et al., 2001), and dynamic precision grip (Ehrsson et al., 2001). Therefore, it can be assumed that the involvement of PMd in down-scaling is not specific to the present paradigm, but generalises across all manual skills requiring a fine and flexible tuning of the motor output.

In everyday life, we do not integrate two conflicting sources of predictive weight information when grasping and lifting an object. The ability to effectively rescale the motor output based on visual cues is more relevant to visually guided activities required by human-machine systems, for instance the manipulation of tools during minimally invasive surgery or performing a landing with an airplane.

In contrast to predictive force scaling based on arbitrary visual cues, cTBS 80% over left PMd did not influence anticipatory force scaling based on the weight of the previous grip. Despite of the presence of a visual S1 pre-cue, the motor system still implemented the somatosensory information about the weight of the previous lift in anticipatory grip force control. This mechanism was not modified by premotor cTBS, suggesting that the stimulated left PMd does not play a crucial role in anticipatory grip force control based on the somatosensory information obtained during the previous lift. This notion is in good agreement with a previous TMS study (Chouinard et al. J Neurosci 2005) in which inhibitory 1Hz rTMS of left M1_Hand but not 1Hz rTMS of PMd impaired predictive scaling of forces based on information acquired during a previous lift. Together, these findings suggest two complementary mechanisms of anticipatory grip force scaling based on arbitrary visual or somatosensory inputs with a selective involvement of the PMd in the former and the M1_Hand in the latter.

**Re-distribution of preparatory activity within premotor areas**

The magnitude of the disruptive effect of cTBS 80% over left PMd on predictive down-scaling of grip force correlated with a shift in preparatory S1-S2 activity in left SMA: cTBS 80% did not affect predictive down-scaling, when SMA increased its preparatory activity for light-weight lifts (relative to heavy-weight lifts) after cTBS 80%. Conversely, cTBS 80% disrupted predictive down-scaling, when SMA was unchanged after cTBS 80%.

The putative role of the rostral SMA in motor control makes this region a plausible candidate for functional compensation: the rostral SMA is critical to conditional response selection based on learned rules (Sakai et al., 1999, Kurata et al., 2000, Donohue et al., 2008). It shows sustained activity during tasks requiring delayed rule-based responses and is engaged in producing appropriate and withholding inappropriate motor responses according to these rules (Mostofsky and Simmonds, 2008), including the re-programming of actions based on changes in conditional response rules (Neubert et al., 2010). We therefore propose that in this study, a relative increase in preparatory activity of rostral SMA effectively compensated for the lesion effect induced in left PMd. This finding speaks against a strict functional segregation between lateral and medial premotor areas in predictive motor control. It suggests a gradual functional differentiation which enables the motor system to maintain functional integrity in the presence of a focal lesion by re-distributing neural activity between medial and lateral premotor areas.

Our results further show that the ability to recruit the rostral SMA varied from subject to subject, resulting in a variable behavioural deficit. This observation highlights the potential of a combined neuromaging-rTMS approach to identify individual differences in functional reorganisation at behavioural level.

**Acknowledgements**

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References


Compensatory activity in the extrastriate body area of Parkinson’s disease patients

BFL van Nuenen, RC Helmich, N Buenen, BPC van de Warrenburg, BR Bloem and I Toni

Abstract

Compensatory mechanisms are a crucial component of the cerebral changes triggered by neurodegenerative disorders. Identifying such compensatory mechanisms requires at least two complementary approaches: locating candidate areas using functional imaging; and showing that interference with these areas has behavioural consequences. Building on recent imaging evidence, we here use this approach to test whether a visual region in the occipito-temporal cortex - the extrastriate body area - compensates for altered dorsal premotor activity in Parkinson’s disease (PD) during motor-related processes. We separately inhibited the extrastriate body area and dorsal premotor cortex in 11 PD patients and 12 healthy subjects, using continuous theta burst stimulation. Our goal was to test whether these areas are involved in motor compensatory processes. We used motor imagery to isolate a fundamental element of motor planning, namely subjects’ ability to incorporate the current state of their body into a motor plan (mental hand rotation). We quantified this ability through a posture congruency effect, i.e. the improvement in subjects’ performance when their current body posture is congruent to the imagined movement. Following inhibition of the right extrastriate body area, the posture congruency effect was lost in PD patients, but not in healthy subjects. In contrast, inhibition of the left dorsal premotor cortex reduced the posture congruency effect in healthy subjects, but not in PD patients. These findings suggest that the right extrastriate body area plays a compensatory role in PD by supporting a function that is no longer performed by the dorsal premotor cortex.

Introduction

Neurodegenerative disorders are often associated with system-level compensatory phenomena, with behavioural impairments emerging from a cerebral balancing act between compensatory and degenerative processes: neurodegeneration starts several years before affected individuals start to display clinically visible symptoms, allowing for cerebral compensation to develop in unaffected brain areas (Braak et al., 2003, Palop et al., 2006). Characterizing cerebral compensatory phenomena is crucial for their potential therapeutic exploitation, and for understanding how different cerebral circuits can support the same function or yield the same output (Edelman and Gally, 2001).

Parkinson’s disease (PD), a prototypical example of a neurodegenerative disorder, is characterized by degeneration of dopaminergic neurons in the substantia nigra pars compacta. This degeneration causes dopamine depletion and disrupts the function of both the basal ganglia and the connected neural circuits, e.g. the basal ganglia-thalamo-motor cortex circuit (Blandini et al., 2000). Disruption in this circuit eventually leads to motor deficits, clinically apparent as the classic symptoms of PD: akinesia, hypokinesia and bradykinesia (Berardelli et al., 2001). Observations in animal models of PD and in clinically unaffected humans at risk of developing PD (i.e. asymptomatic Parkin and PINK1 mutation carriers) have shown that, before the disease becomes symptomatic, compensatory mechanisms arise within the fron-to-striatal circuit (Bezard et al., 2003, Buhmann et al., 2005, van Nuenen et al., 2009). After clinical signs have become evident, compensatory mechanisms appear to engage circuits involving more posterior sensory regions (Sabatini et al., 2000, Helmich et al., 2007). These changes might explain why PD motor deficits improve when external sensory cues are provided (Suteerawattananon et al., 2004, Azulay et al., 2006, Keus et al., 2009). Accordingly, we have recently shown that the right extrastriate body area (EBA), a visual region in the occipito-temporal cortex, showed stronger activity and connectivity with the left dorsal premotor cortex (PMd) when PD patients imagined moving their most-affected hand (Helmich et al., 2007). However, it remains difficult to prove that stronger EBA connectivity (as identified using functional imaging) is compensatory, rather than a collateral by-product of basal ganglia disinhibition. Further supportive evidence requires at least a second complementary approach: showing that interference with the candidate area identified by functional imaging has behavioural consequences.

We have used this approach to test the possibility that the EBA compensates for altered PMd activity in PD during motor-related processes. Specifically, we have used continuous Theta Burst Stimulation (Huang et al., 2005) to selectively inhibit
either EBA or PMd to test their possible compensatory role in PD. We assessed the consequences of this interference on both corticospinal excitability and cerebral motor function. The latter was indexed through a validated motor imagery task that quantifies subjects’ ability to incorporate the current state of their body into a motor plan (de Lange et al., 2006, Helmich et al., 2007). This ability should be reduced if EBA or PMd play a compensatory role in PD.

Materials and Methods

Subjects

Eleven PD patients (6 men, 52.0 ± 7.8 years, mean ± S.D.; table 1 for clinical characteristics) and twelve healthy subjects (6 men, 61.3 ± 6.4 years; mean ± S.D.; t(21) = 2.912; p = 0.01) participated after giving informed consent according to institutional guidelines of the local ethics committee (CMO region Arnhem-Nijmegen, the Netherlands). Participants were consistently right-handed according to the Edinburgh handedness inventory (Oldfield, 1971). Before the experiment, the patients’ disease severity was assessed by one examiner (BFLvN) using the Hoehn and Yahr stages and the Unified Parkinson’s Disease Rating Scale (UPDRS; table 1). Patients were included if they had idiopathic PD, diagnosed according to the UK Brain Bank criteria by an experienced movement disorder specialist (BRB), with clearly right-lateralized PD symptoms. Exclusion criteria were: cognitive dysfunction (i.e. mini mental state examination <24), other neurological diseases (such as severe head trauma or stroke) and general exclusion criteria for TMS (such as epilepsy, pace-maker, implanted metal parts and cardiac arrhythmias). Patients were all studied twice with at least a one-week interval and off-medication with at least a 12 h withdrawal of medication (i.e. practically defined off-condition (Langston et al., 1992)). The whole experimental protocol involved 2 experimental sessions, with session order counterbalanced between participants. The experimental sessions were identical apart from the cTBS protocol which used either cTBS over the right EBA or the left PMd. At least seven days elapsed between the two sessions to exclude carry-over effects of TBS conditioning. Fig.1 (panel A) illustrates the order of the experimental procedures. At the beginning of each experimental session, participants were trained on the motor imagery task (192 trials and <10% of errors, see below for task description). Afterwards, cortical excitability of the left primary motor hand area (M1) was probed with single-pulse TMS of left M1, recording motor-evoked potentials (MEPs) from the right first dorsal interosseus (FDI) muscle. Following performance of a set of 384 motor imagery trials, cortical excitability in left M1 was measured again. Following application of cTBS to either the left PMd or the right EBA (40 seconds), participants rested for seven minutes without moving their hands or feet. We introduced this resting period because previous studies have shown that short periods of voluntary motor activity shortly before or after cTBS can strongly modulate the conditioning effects of cTBS on corticospinal excitability (Huang et al., 2005, Gentner et al., 2008). Following this resting period, cortical excitability in left M1 was measured again to capture acute TBS-induced changes in corticospinal excitability. Thereafter, participants performed the motor imagery task again (384 trials). The experimental session was completed with a fourth measurement of cortical excitability with single-pulse TMS over the left M1.

Motor imagery task

We asked participants to perform a hand laterality judgment task, i.e. a task that has been repeatedly shown to reliably evoke motor imagery (de Lange et al., 2006, Helmich et al., 2007). Participants were shown line drawings of one hand at a time, either left or right, with either the back or the palm of the hand in view. The left and

Table 1 Clinical characteristics

<table>
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<tr>
<th>Patient</th>
<th>Gender</th>
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<th>UPDRS-L</th>
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Mean 6 men 52 1.4 1.1 7.6
S.D. 8 0.5 1.3 3.1

Eleven patients (6 men; age 52.0±7.8 years; mean±S.D.) with idiopathic Parkinson’s disease were tested in a practically defined off-state (i.e. more than 12 hours after having taken their last medication). All patients were consistent right-handers. Patients had markedly asymmetric symptoms lateralized to the right side of their body. UPDRS: Unified Parkinson’s Disease Rating Scale; H&Y: Hoehn and Yahr Rating Scale; M: man; W: woman.
right hand drawings were identical mirror images. A hand drawing could be shown rotated from its upright position in either a counter-clockwise (CCW) or a clockwise (CW) orientation. For both orientations, four different rotations from 45° to 135° in steps of 30° were used, yielding eight different rotations. The stimuli were presented through a PC running Presentation software (Neurobehavioral systems, Albany, USA). They were projected onto a screen in front of the subjects. The subjects’ task was to report whether the hand drawing on display represented a left or a right hand by pressing one of the two buttons with the corresponding left or right foot. During the task, reaction times and error rates were measured. One experimental session consisted of 32 blocks. Each block consisted of 12 trials, which started with a fixation cross displayed for a variable interval (0.5-1.5 s), followed by the presentation of a hand drawing. After a response was provided (reaction time cut-off: 5 sec), the stimulus was replaced by the fixation cross for a jittered period of 0.5-1.5 seconds and then the next hand drawing was shown. On each trial, a different hand drawing was presented, pseudorandomly sampled from a set of 32. During the experiment, the posture of the subjects’ left and right hand was manipulated. At the beginning of each block, a text instructed the subjects to position their arms in one of four postures: (1) both hands with the palm up; (2) left hand palm up, right hand palm down; (3) left hand palm down, right hand palm up; (4) both hands palm down. The period during which this instruction was displayed had a fixed duration (5 sec) and was followed by a block of 12 trials. The posture effect was established by comparing the presented hand drawing and the actually instructed posture of the subject. The posture for a trial was coded as “matching” when the side of the hand (palm or back) and the laterality (left or right) of the hand drawing corresponded with the hand position of the subject. We also considered the influence of biomechanical constrains (BMC) on motor imagery performance. BMC were operationalized as the RT difference in mentally rotating a hand towards either an orientation lateral to the body axis (i.e. towards the extreme range of movement allowed by the arm joints) or towards an orientation medial to the body axis (i.e. towards a comfortable arm configuration). Lateral and medial orientations were coded as follows: CCW rotations (-135°, -105°, -75° and -45°) were averaged and recoded as a lateral orientation for left hands and a medial orientation for right hands; CW rotations (45°, 75°, 105°, 135°) were averaged and recoded as a medial orientation for left hands and a lateral orientation for right hands. Participants were seated in front of the screen with their hands in a box (75 x 27 x 9.5 cm), so there was no visual feedback of the own hands. Before the start of the experiment, patients were trained until they could perform the task with an accuracy of at least 90% correct responses.
Continuous theta burst stimulation of left PMd and right EBA

We used cTBS for conditioning of left PMd and right EBA because cTBS has been shown to produce a lasting suppression of regional excitability in the stimulated cortex. (Huang et al., 2005). The cTBS protocol involved repeated administration of short high-frequency bursts. Each burst consisted of three pulses given at an inter-stimulus interval (ISI) of 20 ms (corresponding to a rate of 50 Hz). These high-frequency triple-pulse bursts were repeated every 200 ms. Theta burst stimulation was given to left PMd as a continuous train lasting for 40 s. The intensity of cTBS was set at 80% of the individual AMT. In every subject, cTBS was delivered over the right EBA and over the left PMd in two different sessions with a minimal interval of seven days. The location in the first session was randomized, so in the first session half of the subjects and half of the patients had cTBS over the right EBA and the other subjects had cTBS over the left PMd. The site for left PMd stimulation was 2 cm anterior and 1 cm medial to the “motor hot spot” on the left motor cortex. The site for right EBA stimulation was determined on the basis of the following procedure. We performed a pilot in 5 subjects (4 men), in which the stereotactic coordinates of the right EBA, as obtained in Helmich et al. (Helmich et al., 2007) (MNI coordinates: \([x\ y\ z] = [-46\ -78\ +6]\) were mapped onto T1 structural scans of those subjects. Each individual right EBA location was then projected on the skull with a stereotactic image guidance system (Brainsight, Rogue Research, Montreal, Canada). This procedure revealed that the skull projection of the right EBA had a consistent location across the five subjects, namely 12 cm laterally and 7 cm posterior to the vertex (Cz). Accordingly, we used this skull-based coordinates to localize EBA in the participants tested in this study. For both simulation sites, the coil was positioned tangentially to the skull with the handle pointing backwards and laterally at an angle of approximately 45° to the sagittal plane (Urgesi et al., 2004, Urgesi et al., 2007).

Analysis of task performance

We considered two outcome measures of the effects of cTBS on PMd and EBA: one behavioural and one physiological outcome measure. The behavioural outcome was performance of the motor imagery task, and more specifically how motor features like biomechanical constraints and the current hand posture influenced reaction times. Behavioural data were analyzed using SPSS Statistics 19.0. Incorrect responses (either wrong or no response) were excluded from further analysis. We considered reaction times (RT) of the correct responses (measured in ms) and number of errors (ERRORS, i.e. errors/total number of trials(%)). ERRORS and RT from both EBA and PMd pre-intervention sessions within each group were combined as a single baseline (paired-samples t-test on ERRORS and RT on these sessions did not reveal any significant difference). First, we tested whether participants from the two groups performed the motor imagery task appropriately (before receiving cTBS), and whether they did so in a comparable manner. We analysed the influence of the factors GROUP (2 levels: CONTROL or PATIENT), LATERALITY (2 levels: RIGHT or LEFT), ORIENTATION (2 levels: LATERAL or MEDIAL), POSTURE (2 levels: MATCH or NONMATCH) and ROTATION (8 levels: from -135° to -45° and 45° to 135° in steps of 30°), by means of a five-way repeated measures ANOVA on RT collected during the baseline sessions. Second, we analysed how cTBS over EBA or PMd influenced overall reaction times and error rates, by assessing the effects of factors GROUP (2 levels: CONTROL or PATIENT), SESSION (3 levels: baseline, post-EBA session, post-PMd session) by means of two two-way repeated measures ANOVA’s on errors and RTs. Third, we tested separately the effect of biomechanical constraints (BMC) and hand posture (HP) on behavioural performance. Specifically, we tested the effect of factors GROUP (2 levels: healthy subjects or patients), SESSION (3 levels: baseline, post-EBA session or post-PMd session) and POSTURE (match or non-match) or ORIENTATION (lateral or medial) on the RT using two three-way repeated measures ANOVA’s. The Greenhouse–Geisser method was used to correct for non-sphericity. Alpha-level was set at \(p = 0.05\).

Measurement and analysis of corticospinal excitability

The physiological outcome measure of cTBS effects was corticospinal excitability, assessed with single-pulse TMS over the left M1 using a biphasic pulse configuration and a 70 mm diameter figure-of-eight shaped coil (Magstim Company Ltd., Whitland, Wales) connected to a Magstim Super Rapid stimulator (Magstim Company Ltd., Whitland, Wales). TMS was applied while participants were comfortably seated in an armchair. Both arms were supported by a cushion to facilitate complete relaxation of the arm and hand muscles. Subjects were instructed to relax but to keep their eyes open and fixate on a wall 1.5 meters in front of them. The coil was positioned tangentially to the skull over the left M1, with the handle pointing backwards and laterally at an angle of approximately 45° to the sagittal plane. At this coil orientation, the second phase of the biphasic TMS pulse induces an electrical current in the brain tissue with a posterior-lateral to anterior-medial direction roughly perpendicular to the central sulcus which is optimal for evoking a motor response in the contralateral hand (Mills et al., 1992). We defined the scalp site where a single TMS pulse at slightly suprathreshold intensity consistently yielded maximal Motor Evoked Potential (MEP) in the right contralateral FDI muscle. This “motor hot spot” was used as stimulation site for all TMS measurements and used as anchor point to define the site for TBS of the left
Results

Patients

The patients had markedly lateralized symptoms according the UPDRS (two-samples t-test: t(10)=9.8; p<0.001; table 1) and were in average 9.3 years younger than the healthy subjects (t(21)=2.9; p=0.01).

Behavioural performance at baseline

Overall performance: Patients and healthy subjects performed the task accurately, with mean error rates and mean reaction times over all sessions that were comparable across groups (mean error rates ± S.E.M.: healthy subjects: 2.3±0.7%; patients: 3.3±1.1%; 2-samples t-test: t(21)=−0.407; p=0.688; mean RT± S.E.M. healthy subjects: 1257±81 ms; patients: 1194±97 ms; 2-samples t-test: t = 0.505; p = 0.619).

Mental rotation performance: RTs changed as a function of stimulus rotation (main effect of ROTATION: F(7,19)=32.38; p<0.001) and this effect was comparable across groups (interaction GROUP X ROTATION: F(7,2)=0.40; p=0.636) as well as EMG data recording, storage, and analyses was performed with Spike2 software (Cambridge Electronic Design, Cambridge, UK). To measure corticospinal excitability we applied 20 pulses with a mean of 0.2 Hz (with random interstimulus intervals of 4, 5 or 6 seconds) and an intensity necessary to obtain a 1 mV MEP in the contra-lateral FDI. After baseline-recording this was repeated four times, before and after each motor imagery task (Fig 1; panel B).

Peak-to-peak amplitudes (mV) of the MEP recorded from the right FDI muscle were measured trial-by-trial and mean MEP amplitudes were calculated for each block of measurements (MATLAB software, Mathworks, Natick, USA). First, we conducted a paired-samples t-test for the MEPs for each group between the two pre-intervention sessions. When there were no significant differences between the two pre-intervention sessions we further combined them as one baseline mean for each group and each intervention. Repeated measures ANOVA was used to test for the main effects of cTBS over left PMd or right EBA on excitability of ipsilateral left PMd. To individually adjust the stimulus intensity, we determined the resting and active MT. We first determined the resting MT in the relaxed FDI muscle which was defined as the minimum stimulus intensity that produced an MEP of more than 50 μV divided by 5 in the last 10 consecutive trials. We then measured the active MT defined as the lowest stimulus intensity at which MEPs were elicited in the last 5 out of 10 consecutive trials during tonic contraction of the FDI muscle at about 10% of maximum force level using a criterion for the MEP of 100–250 μV peak-to-peak amplitude. MTs were determined by gradually decreasing and increasing the stimulus intensity in steps of 1% of maximum stimulator output.

MEPs were recorded with surface electromyography (EMG). Ag–AgCl disc surface electrodes were attached over the right FDI muscle using a belly-tendon montage. The grounding electrode was placed at the wrist. Electromyographic activity was continuously monitored using visual (oscilloscope) and auditory (speakers) EMG feedback to ensure either complete relaxation at rest or a constant level of EMG activity during tonic contraction. The raw EMG signals were filtered between 20 and 1000 Hz, and digitized at 5000 Hz per channel (A/D converter; model Micro1401, Cambridge Electronic Design, Cambridge, UK). The administration of TMS pulses as well as EMG data recording, storage, and analyses was performed with Spike2 software (Cambridge Electronic Design, Cambridge, UK). To measure corticospinal excitability we applied 20 pulses with a mean of 0.2 Hz (with random interstimulus intervals of 4, 5 or 6 seconds) and an intensity necessary to obtain a 1 mV MEP in the contra-lateral FDI. After baseline-recording this was repeated four times, before and after each motor imagery task (Fig 1; panel B).

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Behavioral changes induced by cTBS

Overall performance: There were no significant between groups differences in error rates and RT before the cTBS intervention (baseline session). The mean error rate did not significantly change between sessions (main effect of SESSION: F(2,18)=4.29; p=0.032) and there were no significant between-sessions differences across groups (SESSION X GROUP interaction: F(2,15)=0.013; p=0.950). The mean RT changed between sessions (main effect of SESSION: F(2,19)=18.39; p<0.001), indicating a time-related effect that, importantly, did not differ between groups (GROUP X SESSION interaction: F(2,19)=0.43; p=0.653; Fig 2; panel B).

Mental rotation performance: To assess whether cTBS altered movement-related processes, we considered how biomechanical constraints (BMC) and hand posture (HP) influenced cTBS effects on task performance. Given the presence of time-related effects across sessions (see previous paragraph), we focused these
analyses on differential RT effects sampled within the same experimental session. For each session, we considered the RT differences between lateral and medial hand orientations (BMC ΔRT) and the difference in RT between matching and non-matching hand postures (HP ΔRT). We found that cTBS over either EBA or PMd had opposite effects on the ability of patients or healthy subjects to incorporate their hand posture into imagined movements (GROUP x SESSION interaction on HP ΔRT: F(2,18)=7.52; p=0.005; Fig. 3). Paired-samples t-test revealed that in the PD group, EBA-cTBS significantly reduced the effect of hand posture on imagery performance in the PD group as compared to baseline (HP ΔRT: t(10)=2.55; p=0.029). In the control group, PMd-cTBS significantly reduced the effect of hand posture on imagery performance as compared to EBA-cTBS (HP ΔRT: t(11)=2.29; p=0.043; Fig 3). This interaction was not driven by a speed-accuracy trade-off in the posture effects (GROUP x SESSION x POSTURE interaction on error rate: F(2, 20)=0.997; p=0.385).

Figure 2 Behavioural performance.
(A) Response times (mean±S.E.M.) during the baseline sessions (marked as “pre” in table 1) as a function of group (healthy subjects or Parkinson disease patients) and stimulus rotation (as illustrated by the hand drawings). Response times changed as a function of stimulus rotation for both groups. (B) Response times (mean±S.E.M.) as a function of group (healthy subjects or Parkinson patients) and experimental session (baseline, after cTBS over EBA, and after cTBS over PMd). Response times decreased after either cTBS interventions, similarly for both groups.

Figure 3 Behavioural performance: effects of cTBS.
Panel A: Differences in response times (RT; mean in ms±S.E.M.) between trials with non-matching and matching configurations between subjects’ own hands and hand drawings on display (posture congruency effect). Data are shown as a function of group and experimental session. cTBS over PMd reduced the posture congruency effect in the healthy control group. cTBS over EBA reduced the posture congruency effect in the PD group.
There were no significant between-group differential effects of cTBS intervention when considering biomechanical constraints (GROUP x SESSION interaction on BMC ΔRT: F(2,22)=0.056; p=0.878). These findings were confirmed in additional analyses performed on the absolute RTs (values shown in Table 2), showing that cTBS over either EBA or PMd had opposite effects on the ability of patients or healthy subjects to incorporate their hand posture into imagined movements (GROUP x SESSION x POSTURE interaction on RT: F(2,20)=17.78; p<0.001).

Paired-samples t-test revealed that in the PD group, EBA-cTBS diminished the effect of hand posture on imagery performance in the PD (HP (match vs non-match): t(10)=-1.16; p=0.272) as compared to PMd-cTBS (HP (match vs non-match): t(10)=3.27; p=0.008) and baseline (HP (match vs non-match): t(10)=3.60; p=0.005). In the control group, PMd-cTBS diminished the effect of hand posture on imagery performance (HP (match vs non-match): t(11)=1.65; p=0.127) as compared to EBA-cTBS (HP (match vs non-match): t(11)=3.13; p=0.010) and baseline (HP (match vs non-match): t(11)=3.97; p=0.002). Again, there were no significant between-group differential effects of cTBS intervention when considering biomechanical constraints (GROUP x SESSION x ORIENTATION interaction on RT: F(2,20)=0.056; p=0.878).

Finally, there were no significant between sessions effects (SESSION on BMC ΔRT: F(2,18)=3.06; p=0.087; SESSION on HP ΔRT: F(2,20)=2.53; p=0.107) or laterality effects (HAND on BMC ΔRT: F(1,10)=1.06; p=0.328; HAND on HP ΔRT: F(1,10)=0.12; p=0.773). There were no significant interactions of HAND with other factors (p>0.1 for all interactions).

It is possible that the altered task performance observed after cTBS over EBA in the PD group could be driven by an impairment of recognizing whether the hand picture shows a palm or a back view, rather than a visuomotor impairment. We tested this possibility with a three-way repeated measurement ANOVA with the factors Group (healthy subjects or patients), Session (baseline or EBA or PMd) and hand orientation (Back or Palm) on RTs. These three factors did not significantly interact (F(2,220) =0.74; p=0.463) indicating that cTBS over EBA does not differentially impair the recognition of palm or back-views of hands in the two groups of subjects.

**Motor Evoked Potentials (MEP)**

The two baseline MEP measurements before cTBS were not significantly different within each of the two groups. This indicates that performing the task did not influence corticospinal excitability of the left M1. In both groups (PD and healthy subjects), and for both sites of stimulation (EBA and PMd), cTBS lead to an initial reduction of corticospinal excitability that was followed by increased corticospinal excitability after motor imagery performance (F(2,17)=5.0; p=0.023). Post-hoc paired-samples t-test revealed a significant decrease in MEPs for the PD group in the first post-PMd session compared to the baseline measurements, but not for the healthy healthy subjects (healthy subjects: p=0.195; PD patients: p=0.027; Fig. 4) and an increase in MEPs for the healthy control group after the motor imagery task after cTBS over PMd compared to the baseline measurements, but not for the PD patients (healthy subjects: p=0.028; PD patients: p=0.119; Fig. 4). There were no significant differences after cTBS over EBA for both groups.

**Discussion**

We assessed a possible compensatory role of the EBA during motor imagery in PD patients, as suggested by a previous fMRI study (Helmich et al., 2007), by testing whether inhibition of this area (using cTBS) influences behavioural performance in PD. This was done by using a validated task that quantifies subjects’ ability to consider their current body posture when imagining a movement (de Lange et al., 2006, Helmich et al., 2007). There are two main results. First, after inhibition of the right EBA, PD patients were unable to benefit from knowledge of their hand posture...
during motor imagery, as indicated by absence of a posture congruency effect (de Lange et al., 2006). The same intervention had no effect in healthy subjects. This suggests that, unlike healthy subjects, PD patients depend upon EBA for providing the motor system with an estimate of the current state of the body in space (derived from somatosensory information, given that subjects could not see their hands during the experiment). Healthy subjects did not require this compensatory activity from EBA or, alternatively, engaged other brain regions to compensate for the transient EBA alteration. Second, inhibition of the left PMd reduced the posture congruency effect in healthy subjects, but not in PD patients. We infer that, in PD, the PMd is functionally disconnected from the cerebral network incorporating the current state of the body in space into a motor plan. The right EBA apparently compensates for this PMd alteration, and this was supported by our finding that overall imagery performance was comparable between patients and healthy subjects.

A compensatory role for the extrastriate body area in PD

Inhibiting the right EBA prevented PD patients, but not healthy subjects, from integrating current estimates of the body state into a motor plan. This effect was not a consequence of EBA-driven changes in corticospinal excitability in either group. Yet, the present findings clearly indicate that the EBA can play a role in motor control, adding causal evidence to previous suggestions (Astafiev et al., 2004, Kuhn et al., 2011). More precisely, we show that PD patients use the EBA to estimate the current state of the body in space, a necessary requirement for specifying a motor plan suitable to achieve a desired end state (Shadmehr and Krakauer, 2008). It remains unclear how the EBA of PD patients can support this function. One possibility is that the EBA estimates the difference between desired and current body posture, integrating visual and somatosensory information (Zimmermann et al., 2011). In our study, subjects had no visual information about the current orientation of their hands, so PD patients would use the EBA for processing somatosensory information about body parts. However, it is unclear whether the EBA receives somatosensory information. Another possibility is that the EBA effects shown in this study reflect remote alterations conveyed from the EBA into parietal and premotor regions known to be involved in estimating the spatial configuration of the body (de Lange et al., 2006, Helmich et al., 2007). In this scenario, compensatory effects of EBA would arise from enhanced connectivity, rather than enhanced local activity.

**Dorsal premotor functionality in PD**

Inhibiting the left PMd prevented healthy subjects, but not PD patients, from integrating current estimates of the body state into a motor plan. We draw three inferences from these observations. First, the left PMd of PD patients was physio-
logically sensitive to cTBS (as indicated by an MEP reduction), but that intervention did not influence motor imagery performance. This finding suggests that the known hyperactivity of premotor areas in PD (Sabatini et al., 2000; Wu and Hallett, 2005) is more likely to be dysfunctional than compensatory in nature. Second, unilateral inhibition of the left PMd in healthy subjects is sufficient to alter their ability to incorporate the current state of their body into a motor plan, a strong confirmation of the known hemispheric dominance of this frontal region for supporting motor imagery (Haaland et al., 2004, de Lange et al., 2006, de Lange et al., 2008). Third, healthy subjects could not recruit compensatory circuits to supplement PMd alterations, as observed in PD patients. This observation suggests that the EBA-based compensatory mechanism found in PD might require time to develop. It is also possible to speculate that the compensatory role of the EBA becomes effective only once the PMd is functionally disconnected from the posterior parietal regions supporting the incorporation of the current body posture into a motor plan (de Lange et al., 2006).

Interpretational issues
Matched performance between patient and control groups is an important pre-condition for isolating compensatory mechanisms (Price and Friston, 2002). In this study, both performance and cTBS effects on cortical excitability were matched across groups at baseline. There was a significant difference in age between groups, and it is possible that control subjects were more sensitive to cTBS stimulation over PMd than PD patients. Elderly subjects might have higher cerebral activation to compensate for age-related decline in functionality (Ramsøy et al., 2011). However, it is unclear how the age difference alone could account for the double dissociation between behavioural consequences of cTBS on EBA and PMd across groups.

It might be argued that motor imagery is a loosely defined phenomenon that could be solved using a variety of strategies and cerebral mechanisms. In fact, the characteristics of the imagery task used in this study allow for specific inferences. Reaction times increased with increasing stimulus rotation for both hands, indicating that the participants used mental rotation to solve the task, in line with previous findings in healthy subjects (Parsons, 1987, 1994, de Lange et al., 2006) and PD patients (Dominey et al., 1995, Helmich et al., 2007). Reaction times were also sensitive to orientation of the stimulus with respect to the body axis, indicating that the participants imagined a movement with the same biomechanical constraints as their own hand. Moreover, reaction times were sensitive to the congruency between the orientation of the hand shown on the screen and the current posture of the subject’s (unseen) hand, indicating that the computations occurring during motor imagery incorporated the current state of the body.

This study builds on recent findings (Helmich et al., 2007), yet the two studies report different effects. However, these studies differs in a number of procedures, and those inconsistencies are likely related to different sensitivities of the outcome measures to different factors influencing performance of the imagery task. Helmich et al. (2007) focused on the effects of biomechanical constraints on metabolic indexes of cortical activity and connectivity. In contrast, the present study was designed for isolating behavioral effects related to the current body posture. Subjects changed their hand posture every 12 trials. This procedure reduced the chances of habituation of the posture congruency effect, and generated differential reaction time effects between posture-matched and un-matched conditions that were un-affected by time-related effects across experimental sessions. By the same token, this measure does not allow us to specify whether the present findings result from increased reaction times in the posture-matched condition or from decreased reaction times in the posture-unmatched condition.

Another outstanding issue concerns the interference procedure, and in particular the largely unknown physiological effects of cTBS on diseased brains. Continuous TBS produces inhibitory effects when applied over different cortical areas (Franca et al., 2006, Huang et al., 2009, Volman et al., 2011). In this study, MEPs decreased immediately after cTBS over PMd in both the PD group (statistically) and in healthy subjects (numerically). Similarly, a recent study demonstrated that cTBS given to the right PMd of healthy subjects did not change the size of MEPs recorded from either left or right abductor pollicis brevis muscle (Stefan et al., 2008). In this study, MEPs increased in both healthy and PD patients (numerically) after cTBS was followed by ~25 min of motor imagery. This effect fits with the well-known state-dependency of cTBS effects. For instance, one minute of voluntary contraction of a muscle, during or after cTBS, changes the size and direction of the TBS after-effects (Huang et al., 2008). This study suggests that motor imagery evokes similar cTBS after-effects as voluntary contractions, in line with the neurophysiological overlap between motor imagery and movement execution (Jeannerod and Frak, 1999, Cisek and Kalaska, 2004).

Conclusion
We have shown that patients with PD use a visual cortical area, the extrastriate body area, to influence the motor imagery network that encodes the current state of the body in space during the generation of a motor plan. This compensatory effect might be related to altered PMd functionality in PD, and it might be implemented through changes in long-range connectivity between EBA and PMd (Helmich et al., 2007). These findings provide causal evidence for the compensatory
role of a visual cortical region during motor-related processes in PD, opening the way for understanding how the extrastriate body area can improve motor function in this disorder, and whether this improvement is directly related to dopaminergic dysfunction.

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References


Summary, outlook and future perspectives
Summary and outlook

This chapter discusses the main findings of this thesis, which aimed to provide different examples of cerebral reorganization in healthy controls and in subjects with premotor parkinsonism. We used a multimodal imaging approach, combining functional Magnetic Resonance Imaging (fMRI) and transcranial magnetic stimulation (TMS). Changes were observed both at the behavioral level (e.g., changes in grip force rate or reaction times) and locally in specific brain regions (e.g., altered task-related activity in the or extrastriate body area). In the next paragraphs, I will provide a summary and discussion of these findings. I will conclude this chapter with some perspectives for future research in this field.

Premotor parkinsonism

Next to the influence of environmental factors and aging, there is a strong genetic component to the pathogenesis of PD as described in chapter 2. In the last decade, several monogenetic forms of parkinsonism have been discovered, both recessive and dominant in nature. The recognition of asymptomatic carriers of mutations in these genes makes it possible to do research in these subjects, who are assumed to be in a premotor stage of the disease. Knowledge derived from these studies will give us a better understanding of the pathogenesis of PD, in both the premotor and motor phase of the disease. It is known that mutations in the Parkin (PARK2) and PINK1 (PARK6) genes can cause recessively inherited PD, as is discussed in more detail in chapter 3. The presence of a single Parkin or PINK1 mutation should theoretically not cause PD, but turns out to be associated with a mild but detectable dopaminergic nigrostriatal dysfunction (as can be detected using nuclear imaging of the dopaminergic system). Moreover, carrying such a single mutation in a recessive Parkinson gene conveys a mildly increased risk to develop PD, typically later in life. Therefore, neuroimaging of non-manifesting individuals with a single mutant Parkin or PINK1 allele opens up a window for the in vivo investigation of the premotor and very early phases of PD. Specifically, we outline that fMRI can be used to identify potential compensatory mechanisms that might help to delay development of clinically manifest disease.

Premotor compensatory processes in PD

The first evidence for motor redistribution at a systems level in non-manifesting carriers of a heterozygous mutation in the Parkin gene was provided by fMRI (Buhmann et al., 2005). With internally cued movements, Parkin mutation carriers displayed a stronger activation of the right rostral cingulate motor area (rCMA) and left dorsal premotor cortex (PMd) compared with externally cued movement (Buhmann et al., 2005). They also showed stronger functional coupling between
the rCMA and posterior putamen during internal movement selection. Because mutation and non-mutation carriers performed the task equally well, these activity changes were attributed to an adaptive redistribution that helps to maintain motor function in the context of a latent nigrostriatal dysfunction. Chapter 4 extended this previous study. In the first fMRI study (Buhmann et al., 2005), the onset of each movement was externally paced at a low rate. Hence, movements were not sequentially performed but separated by periods of rest. The focus of that study was on motor circuits subserving the internal selection of each movement as opposed to external movement selection. In our study (chapter 4), participants quickly performed a brief "chunk" of three movements as indicated by external spatial cues. This task mainly probed neuronal circuits subserving the control of movement sequences rather than internal movement selection.

By using a "real" sequential task, we hypothesized that the study as described in chapter 4 would reveal a different regional pattern of motor reorganization relative to the first study in non-manifesting heterozygous Parkin mutation carriers. These regional "discrepancies" would confirm the hypothesis that there is no regionally fixed pattern of motor areas (i.e. no "default" pattern of motor reorganization). Furthermore, we studied adaptive redistribution of cortical activity within a pre-existing motor network in non-manifesting carriers of a single mutant allele in the Parkin or PINK1 gene. This enabled us to examine whether the patterns of compensatory recruitment of areas that subserve complex motor sequences were specifically linked to mutations in a specific gene. Given the closely related dysfunctional effects of mutations in both proteins in a drosophila model (Clark et al., 2006, Park et al., 2006), our prediction was that the functional phenotype at a brain network level would be similar for both groups. We indeed showed that when non-manifesting heterozygous carriers of a Parkin or PINK1 mutation perform a simple motor sequence task, both groups recruit areas that are not utilized by healthy controls without such a mutation, namely the pre-SMA and right rostral PMd. No convincing differences were found between the two groups of mutation carriers. These findings might also be a functional correlate of the increase in grey matter volume in the basal ganglia that was found in a comparable group of non-manifesting carriers of a Parkin or PINK1 mutation using morphometric MRI (Binkofski et al., 2007). Taken together, these functional and structural MRI data suggest that non-manifesting heterozygous mutation carriers of a Parkin or PINK1 gene produce a very similar functional and structural endophenotype. These findings imply that single heterozygous mutations in these two different genes have a similar impact on the human motor system, and suggest the human brain adapts in a 'generic' way to a mild presynaptic dopaminergic lesion, despite differences in specific etiology.

In chapter 5, we extended these findings to a unique, large group of non-manifesting LRRK2 mutation carriers. This study differed from the previous fMRI study (chapter 4) in two crucial ways: first, we used a well-established motor imagery paradigm which excluded the possibility that the altered cerebral activity was due to altered sensory feedback in the non-manifesting mutation carriers (Gierthmuhlen et al., 2010). Secondly, we focused on non-manifesting carriers of a mutation in the LRRK2 gene, which causes dominantly inherited PD and is the most frequent genetic cause of PD, particularly in those of Ashkenazi Jewish origin (Farrer, 2006). Importantly, the risk of developing overt PD is much higher (up to 80% at age of 70 years (Healy et al., 2008)) in these LRRK2 mutation carriers, as compared to the previously studied non-manifesting carriers of a single mutation in a recessive gene (Parkin or PINK1 gene) (Clark et al., 2006, Park et al., 2006). Therefore, these dominant mutation carriers offer a much more powerful model of studying the functional phenotype at a brain network level, and offers further opportunities to study whether this adaptive plasticity is similar to the pattern observed previously in the mutation carriers in recessive genes.

Several groups have actually explored these possibilities. For example, previous studies using transcranial sonography of the substantia nigra in manifesting and non-manifesting LRRK2 carriers showed substantia nigra signal changes, which were not substantially different from idiopathic PD (Bruggemann et al., 2011), suggesting the presence of intermediate nigrostriatal pathology that apparently appears on in the course of parkinsonism. Additionally, voxel based morphometry in 30 asymptomatic mutation carriers of four different genes associated with PD showed an increased grey matter volume mainly in the putamen in asymptomatic Parkin, PINK1 and ATP13A2 mutation carriers, and conversely an increased grey matter volume of the right caudate in the LRRK2 carriers (Reetz et al., 2010).

In chapter 5, we addressed the question whether the latent nigrostriatal dysfunction caused by a mutation in the LRRK2 G2019S gene gives rise to cortical reorganization in the premotor phase, and whether this can be explained by anatomical differences in the human striatum. To this end, asymptomatic first-degree relatives of symptomatic patients with the LRRK2 G2019S mutation underwent high-resolution structural and functional MRI. During fMRI, the subjects performed a well-defined motor imagery task that involved mental rotation of hand pictures presented to the subjects, forcing participants to decide whether the projection displayed a left or right hand. A further manipulation involved a change in the subject’s own hand position during the experiment, because subjects use this position as a reference for mental rotation. We chose this approach since with use of this specific motor imagery paradigm cortical reorganization was previously shown in the right extrastriate
body area (EBA) and left dorsal PMd of patients with clinically overt PD (specifically, during mental rotation of the affected hand in right-sided affected PD patients) (Helmich et al., 2007). The new study presented in chapter 5 yielded three main findings. First, LRRK2-carriers and non-carriers were equally sensitive to the extent and biomechanical constraints of the imagined movements, and to the current posture of their own hands. This result validates the relevance of the motor imagery task: all subjects were effectively engaged in internally selecting motor representations, and any observed cerebral effects could not have been driven by between-groups differences in performance. Second, non-manifesting LRRK2-carriers had reduced imagery-related activity in the right caudate nucleus. This result indicates that non-manifesting LRRK2-carriers already have some form of functional impairment in the striatum. Furthermore, in contrast to the ventrolateral striatal alterations seen in idiopathic PD patients and in premotor carriers of Parkin and Parkin and PINK1 (Binkofski et al., 2007, Reetz et al., 2008), the striatal impairment of non-manifesting LRRK2-carriers arose in the caudate nucleus. Third, non-manifesting LRRK2-carriers had increased imagery-related activity in the right PMd, and increased effective connectivity between this region and the right EBA. This result suggests that the PMd might support compensatory activity through long-range connectivity with posterior sensory regions.

Interference of cortical function using cTBS in healthy controls
The next step after the identification of several premotor regions that play an important compensatory role in premotor parkinsonism, was to demonstrate the compensatory role of these regions by means of functional interferences. This was done with TMS, hypothesizing that inhibition of these regions would lead to measurable impairments in task performance. To determine that such functional interferences can indeed be mapped, we used a conditioning-and-map approach in 11 healthy men. This also allowed us to further clarify the role of PMd in anticipatory motor control (chapter 6), since it is known that the PMd uses prior sensory information for motor preparation (Picard and Strick, 1996, Passingham et al., 1998, Kurata et al., 2000, Toni et al., 2002, Amiez et al., 2006, van Eimeren et al., 2006). We transiently disrupted neuronal processing in left PMd, using continuous theta burst stimulation (cTBS; see figure 1.4). The conditioning effects of cTBS on preparatory brain activity were assessed with fMRI while participants performed a ‘grip-and-lift’ task. The experiments firstly showed that cTBS of left PMd selectively impairs anticipatory downscaling, abolishing the grip force undershoot but not overshoot in trials with incorrect prece. Secondly, in the absence of cTBS, individual variations in preparatory activity of left PMd as triggered by a “light weight” cue predicted the relative grip force undershoot for incorrectly precued heavy-lift trials. Thirdly, this association between preparatory activity in the left PMd and individual variations in grip force undershoot was cancelled by cTBS of the left PMd. Taken together, these results provide converging evidence for a causal involvement of PMd in anticipatory down-scaling of grip force, suggesting an inhibitory role of PMd in anticipatory grip force control during object lifting.

Interference of cortical function using cTBS in Parkinson’s disease
Chapter 6 thus demonstrated that it is indeed possible to capture behavioural and functional changes after inhibiting a specific cortical region with cTBS. We were intrigued by a previous fMRI study that suggested that the EBA might have a compensatory role in PD patients during the generation of a motor plan (Helmich et al., 2007), which was later confirmed by us in non-manifesting LRRK2-carriers (chapter 5). Therefore, we conducted a new experiment where we inhibited this region with cTBS, in order to test whether this area is indeed involved in motor compensatory processes (chapter 7). The consequences of this intervention were assessed with a validated task, which was the same as used in chapter 5 and which quantifies the subjects’ ability to consider their current body posture when imagining a movement (de Lange et al., 2006, Helmich et al., 2007). There were two main results. First, inhibition of the right EBA made PD patients lose the performance benefits associated with having a body posture congruent to the imagined movement (posture congruency effect (de Lange et al., 2006)). The same intervention did not have any effect in a group of healthy subjects, indicating that in healthy subjects either EBA is not involved in providing the motor system with an estimate of the current state of the body in space, or that other brain regions can compensate for the transient EBA disruption. Second, inhibition of the left PMd reduced the posture congruency effect in healthy subjects, but not in PD patients. We infer that in clinically manifest PD, the PMd can no longer be recruited for this specific task and the right EBA compensates for this. In conclusion, the EBA influences the motor imagery network in PD patients that encodes the current state of the body in space during the generation of a motor plan.

Dorsal premotor functionality in healthy controls and Parkinson’s disease
Inhibition of the left PMd with cTBS prevented healthy subjects, but not PD patients, from integrating current estimates of the body state into a motor plan during a motor imagery task. Three inferences can be drawn from these observations. First, the left PMd of PD patients was indifferent to cTBS during motor-related processes, an indication that the known hyperactivity of premotor areas in PD (Sabatini et al., 2000, Wu and Hallett, 2005, Eckert et al., 2006) is more likely to be dysfunctional.
rather than compensatory in nature. Second, unilateral inhibition of the left PMd in healthy subjects is sufficient to alter their ability to incorporate the current state of their body into a motor plan, a strong confirmation of the known hemispheric dominance of this frontal region for supporting motor imagery (Hlustik et al., 2002, Haaland et al., 2004, de Lange et al., 2006). Third, healthy subjects could not recruit compensatory circuits to supplement PMd disruption as observed in PD patients. This suggests that the EBA-based compensatory mechanism found in PD might require time to develop, or that the compensatory role of the EBA becomes effective only once the PMd is functionally disconnected from the posterior parietal regions that support the incorporation of the current body posture into a motor plan (de Lange et al., 2006). Conversely, in the premotor phase of the disease, as studied in non-manifesting PARK-gene mutation carriers, we found an increased reliance on the PMd during simple motor tasks suggesting compensatory over-activity in order to overcome the (mild) nigrostriatal dopaminergic dysfunction.

Previous neuroimaging studies in PD have consistently shown impaired movement-related activation in distinct frontal motor areas in patients tested off their dopaminergic medication (Playford et al., 1992, Jahanshahi et al., 1995, Samuel et al., 1997, Buhmann et al., 2003, Mentis et al., 2003). For instance, the rostral SMA or right dorsolateral prefrontal cortex showed deficient activation during internal selection of movement onset (Jahanshahi et al., 1995) or the type of movement (Playford et al., 1992) in unmedicated PD patients. This relative hypoactivity in frontal motor areas could be partially reversed by dopaminergic therapy (Jenkins et al., 1992, Haslinger et al., 2001, Buhmann et al., 2003). In contrast to symptomatic PD patients, non-manifesting mutation carriers showed no relative decreases in frontal motor areas. It is possible that in these studies, the nigrostriatal dopamine depletion in these asymptomatic mutation carriers was below the threshold causing impaired movement-related activation of frontal motor areas. Alternatively, a normal or compensatory increased level of activation may be maintained by adaptive mechanisms that facilitate movement-related activity in frontal motor areas and effectively compensate for deficient cortical activation via the cortico-basal ganglia-thalamocortical motor loop. Whatever the cause, our findings lend further support to the notion that the clinical manifestation of parkinsonism ultimately results from a failure of compensatory mechanisms to maintain a sufficient level of movement-related activity in premotor areas (Bezard et al., 2003).

**Future perspectives**

Functional neuroimaging studies, as reported in chapters 3 to 5, have given the first insights in the cerebral structures that are involved in premotor compensation strategies of the human brain in order to overcome the latent nigrostriatal dopaminergic dysfunction. The premotor regions seem to play a key role in these compensatory processes, which are more likely task-dependent rather than gene-dependent (chapter 4). An intriguing question is whether these increases in task-related activity in premotor areas persist, increase or attenuate in mutation carriers who are on the brink of developing clinical (motor) signs of parkinsonism. There are at least two competing explanations for the extra recruitment of premotor areas in these non-manifesting mutation carriers. It may be that among the mutation carriers, who have an increased risk for developing PD, the observed premotor changes reflect early premotor cortical alterations, i.e. an early manifestation of subtle alterations within the motor network. This intriguing possibility can only be confirmed through long-term follow-up of these subjects. Another possibility is that the motor network in these mutation carriers is simply different and as such unrelated to future development of PD. The cortical changes observed in these mutation carriers might therefore reflect, at least in part, an endophenotypic marker and not an early biomarker of PD. Longitudinal studies with repeated functional imaging are now needed to assess whether the observed cortical changes are predictive of PD during the premotor phase and if they are, whether these increase or attenuate when subjects develop clinical signs of PD.

When these longitudinal studies indeed show that the reorganization pattern in premotor areas is correlated with early PD and is not merely an endophenotypic marker, then fMRI protocols such as the ones described in this thesis might serve future diagnostic purposes. If we are able to identify subjects at risk for PD, fMRI can be used to determine the activity of regions involved in compensatory processes in the premotor phase. Moreover, quantification of activity within these regional activities during standardized tasks could provide metrics for stratifying the disease state. Such an approach is needed when starting to think about how to design and interpret future trials with, for example, disease modifying drugs or other treatments.

On top of this potential “diagnostic” use and the new insights in the pathophysiology of PD, our results cautiously point to new treatment options for PD, for instance with TMS. In chapters 6 and 7, we used a relatively new rTMS protocol (cTBS) (Huang et al., 2005) to inhibit cortical areas and map the behavioral consequences of the intervention. With this method we showed that the cortical areas found
Previously are indeed compensatory and engaged in generating a motor plan in PD. Future research should explore whether “boosting” these compensatory areas with rTMS protocols leads to improvement of motor and perhaps even cognitive symptoms of PD. We can expect to encounter a few problems on the way. The effects of rTMS are not focal to a particular cortical area, but spread to distant sites. Even at the site of stimulation, TMS as it is currently used activates a mixture of neural populations that use different neurotransmitters and that have different actions. In addition, the effects of rTMS are variable and depend on the prior history of brain activity, the pathological state of the networks stimulated, and any drugs that patients might be taking (Huang et al., 2008). These caveats are, however, not all negative. The spread of after-effects might be a useful way to target deep structures that are not directly accessible to TMS. Also, a mixture of neural effects in a functional circuit occurring both within and at a distance from the area of stimulation could produce a better effect than stimulation of just a small homogeneous population of neurons. A major problem is that most of the work in healthy subjects suggests that the duration of after-effects is short. Repeated daily sessions may lead to longer-lasting effects (Baumer et al., 2003) but, again, the effects will probably only be minor. So it is reasonable to assume that any sustained therapeutic benefit can be achieved? Finally, should we limit the parameters that are used in patient studies to those that have been deemed safe in healthy individuals? Similar to drug trials, we might need to test a wider range of stimulation parameters (“dose finding”) if the benefit to risk ratio is sufficiently high.

References


CHAPTER 8 SUMMARY, OUTLOOK AND FUTURE PERSPECTIVES


Nederlandse samenvatting

In dit hoofdstuk worden de belangrijkste bevindingen van dit proefschrift besproken, welke als doel had verschillende voorbeelden van cerebrale reorganisatie bij gezonde proefpersonen en bij personen met premotorisch parkinsonisme aan te tonen. We gebruikten hiervoor een multimodale aanpak, die bestond uit een combinatie van functionele Magnetic Resonance Imaging (fMRI) en transcraniële magnetische stimulatie (TMS). Veranderingen werden zowel op gedragsniveau (bijvoorbeeld wijzigingen in grijpkracht of reactietijd) als lokaal in specifieke gebieden van de hersenen (zoals veranderde taakgerelateerde activiteit in de dorsale premotorische cortex) waargenomen. In de volgende paragrafen zal ik een samenvatting geven van deze bevindingen, evenals een discussie tegen de achtergrond van de bestaande literatuur. Ik sluit dit hoofdstuk af met een aantal suggesties voor toekomstig onderzoek op dit gebied.

Premotorisch parkinsonisme

Naast de invloed van omgevingsfactoren en veroudering, is er een belangrijke genetische bijdrage aan de pathofysiologie van de ziekte van Parkinson zoals beschreven in hoofdstuk 2. In de afgelopen tien jaar zijn verschillende mono- genetische vormen van parkinsonisme ontdekt, met zowel recessieve als dominante overervingsvormen. De herkenning van asymptomatische dragers met een mutatie in één van deze genen maakt het mogelijk om onderzoek te doen in deze personen, die zich mogelijk in de premotorische fase van de ziekte van Parkinson bevinden. De kennis die verkregen wordt uit deze studies geeft ons een beter begrip ten aanzien van de pathogenese van de ziekte van Parkinson, zowel in de premotorische als in de motorische fase van deze ziekte. Het is bekend dat mutaties in het Parkin (PARK2) gen of het PINK1 (PARK6) gen een erfelijk parkinsonisme kan veroorzaken met een recessief overervingspatroon, hetgeen in meer detail besproken wordt in hoofdstuk 3. De aanwezigheid van één Parkin of PINK1 mutatie zou theoretisch niet leiden tot parkinsonisme, maar blijkt geassocieerd te worden met een milde, maar detecteerbare dopaminerge nigrostriatale dysfunctie (zoals kan worden aangetoond met behulp van nucleaire beeldvorming van het dopaminerge systeem). Bovendien, een enkele mutatie in een recessief Parkinson gen brengt een licht verhoogd risico met zich mee voor het ontwikkelen van parkinsonisme, meestal op latere leeftijd.

Beeldvormend onderzoek bij personen met één mutant Parkin of PINK1 alleel maar zonder klinische kenmerken passend bij de ziekte van Parkinson, vormt een toegang tot in vivo onderzoek naar de premotorische en zeer vroege fase van de ziekte van Parkinson. Functionele MRI kan gebruikt worden om mogelijke cerebrale compensatie-
mechanismen te identificeren, die de ontwikkeling van klinische symptomen van de ziekte vertragen.

Premotorische compensatie in de ziekte van Parkinson
Het eerste bewijs voor cerebrale reorganisatie in asymptomatische dragers van een heterozygote mutatie in het Parkin gen werd aangetoond met behulp van fMRI (Buhmann et al., 2005). Tijdens het uitvoeren van intern geselecteerde vingerbewegingen vertoonden asymptomatische dragers van een Parkin mutatie een sterkere activiteit in het rostrale cingulate gebied (rCMA) en de linker dorsale premotor cortex (PMd) in vergelijking met extern (d.m.v. een aanwijzing) geactiveerde bewegingen. Deze groep toonde ook een sterkere functionele koppeling tussen de rCMA en het achterste deel van het putamen tijdens intern geselecteerde bewegingen. Omdat mutatiedragers en personen zonder mutatie de taak even goed uitvoerden is deze veranderde activiteit toegeschreven aan een motorische reorganisatie die leidt tot een normale functie bij mutatiedragers ondanks de latente nigrostratiale disfunctie. Hoofdstuk 4 is een uitbreiding van deze eerdere studie. In de eerste fMRI studie (Buhmann et al., 2005) was het begin van iedere beweging extreem geactiveerd met een relatief laag tempo. Zo werden de bewegingen niet snel opeenvolgend uitgevoerd, maar van elkaar gescheiden door perioden van rust. Deze studie was gericht op de motore circuits die zorgdragen voor de interne selectie van elke beweging, in tegenstelling tot externe selectie van een beweging. In onze studie, moesten de proefpersonen zo snel mogelijk drie bewegingen achter elkaar uitvoeren: de volgorde werd vooraf aangegeven door externe spatiale aanwijzingen. Deze taak was vooral gericht op neuronale circuits die de controle van de extern aangestuurde sequenties beïnvloeden. Door het gebruik van een “echte” sequentiële taak, was onze hypothese dat de studie zoals beschreven in hoofdstuk 4 een ander regionaal patroon van motore reorganisatie zou laten zien ten opzichte van het eerste onderzoek in asymptomatische heterozygote Parkin mutatiedragers. Het aantonen van deze regionale ‘verschillen’ zou bevestigen dat er geen standaard patroon van motore reorganisatie is. Daarnaast hebben we de adaptieve hervordering van corticale activiteit in een bestaand motorisch netwerk bestudeerd in asymptomatische dragers van een mutant allel in het Parkin of PINK1 gen. Dit stelde ons in staat om te onderzoeken of de patronen van cerebrale compensatie worden gekoppeld aan mutaties in een specifiek gen. Gezien de nauwverwante disfunctionele effecten van de mutaties in beide eiwitten, zoals aangetoond in een Drosophila model (Clark et al., 2006, Park et al., 2006), was onze voorspelling dat het functionele fenotype op cerebraal niveau gelijk zou zijn voor beide groepen.

We hebben aangetoond dat wanneer asymptomatische heterozygote dragers van een Parkin of PINK1 mutatie een eenvoudige motorische sequentiële taak uitvoerden, beide groepen bepaalde cerebrale gebieden meer activeren dan de gezonde controlegroep zonder een dergelijke mutatie. Hierbij gaat het om de supplementaire motorische cortex (pre-SMA) en de rostrale PMd aan de rechterzijde. Er werden geen overtuigende verschillen gevonden tussen de twee groepen mutatiedragers. Deze bevindingen zijn wellicht ook een functioneel correlaat van de toename van het volume van de grijze stof in de basale ganglia, dat gevonden werd in een vergelijkbare groep asymptomatische dragers van een Parkin of PINK1 mutatie met behulp van morfometrische MRI (Binkofski et al., 2007). Al deze functionele en structurele MRI-gegevens wijzen erop dat asymptomatische heterozygote mutatiedragers van een Parkin of PINK1 gen zeer vergelijkbare functionele en structurele endofenotypen bezitten. Deze bevindingen impliceren dat heterozygote mutaties in deze twee verschillende genen een vergelijkbare impact hebben op het motore systeem. Daarnaast suggereren deze bevindingen dat het humane brein zich aanpast in een ‘generieke’ manier wanneer er sprake is van een mild presynaptisch dopaminerge laesie, ondanks verschillen in specifieke etiologie.

In hoofdstuk 5 hebben we bovenstaande bevindingen ook onderzocht in een unieke, grote groep asymptomatische LRRK2 mutatiedragers. Deze studie verschilde van de vorige fMRI studie (hoofdstuk 4) in twee opzichten. Ten eerste hebben we een veelgebruikt “motorische verbeelding” paradigm toegepast omdat de mogelijkheid dat de veranderde cerebrale activiteit te wijten is aan veranderde sensorische feedback in de asymptomatische mutatie dragers uitsluit (Giertzhuhlen et al., 2010). Bij motorische verbeelding stellen proefpersonen zich voor een beweging te maken zonder deze daadwerkelijk uit te voeren. Eerder onderzoek heeft aangetoond dat tijdens motorische verbeelding (grotendeels) dezelfde hersengebieden geactiveerd worden als tijdens echte bewegingen. Het voordeel van motorische verbeelding is dat het relatief gemakkelijk in de scanner te onderzoeken is. Proefpersonen kregen een plaatje te zien van een hand en moesten vervolgens aangeven of het een linker- of rechterhand was. Om deze vraag te kunnen beantwoorden, draaiden de personen in gedachten hun eigen hand in de oriëntatie van het plaatje. Dit proces heet “mentale rotatie” en is een manier om motorische verbeelding te onderzoeken. Ten tweede hebben we ons gericht op asymptomatische dragers van een mutatie in het LRRK2 gen, dat een dominant erfelijk parkinsonisme veroorzaakt en de meest voorkomende genetische oorzaak van parkinsonisme is, in het bijzonder in mensen van Ashkenazi Joodse origine (Farrer, 2006). Belangrijk hierbij is dat de kans op klinische symptomen van parkinsonisme veel hoger is (tot 80% op de leeftijd van 70 jaar (Healy et al., 2008)) bij personen met een mutatie in het LRRK2 gen in vergelijking met asymptom-
tische dragers van een mutatie in één van de recessieve genen (Parkin of PINK1 gen) (Clark et al., 2006; Park et al., 2006). Daarom zijn deze LRRK2 mutatiedragers een veel krachtiger model voor het bestuderen van het functionele fenotype op motorisch netwerk niveau. Daarnaast biedt deze groep de mogelijkheid om te onderzoeken of het patroon van deze motore reorganisatie vergelijkbaar is met het patroon dat eerder waargenomen werd in de Parkin- en PINK1-gen mutatiedragers. Verschillende onderzoeksgruppen hebben deze mogelijkheden al verkend. Bijvoorbeeld is in eerdere studies met behulp van transcraniale echografie van de substantia nigra bij asymptomatische LRRK2 mutatiedragers een veranderd signaal van de substantia nigra aangetoond, die niet wezenlijk verschilde van de veranderingen die met dit onderzoek gevonden worden bij patiënten met de idiopathische ziekte van Parkinson (Bruggemann et al., 2011). Dit suggereert de aanwezigheid van een intermediaire nigrostriatale pathologie die blijkbaar naar voren komt in het beloop van de ziekte. Daarnaast liet “voxel based morphometry” (VBM) in 30 asymptomatische mutatiedragers van vier verschillende genen geassocieerd met parkinsonisme een toegenomen grijze stof volume zien (vooral in het putamen bij asymptomatische Parkin, PINK1 en ATP13A2 mutatiedragers) en een verhoogde toegenomen grijze stof volume van de nucleus caudatus in de LRRK2 mutatiedragers (Reetz et al., 2010).

In hoofdstuk 5, hebben we de vraag gesteld of de latente nigrostriatale disfuncitie veroorzaakt door een G2019S-mutatie in het LRRK2 gen aanleiding geeft tot corticale reorganisatie in de premotorische fase van de ziekte, en of dit kan worden verklaard door anatomi sche verschillen in het striatum. Hiertoe hebben asymptom atische eerstegraads familieleden van symptomatic patiënten met de LRRK2 mutatie een hoge resolutie structurele en functionele MRI ondergaan. Tijdens fMRI gen aanleiding geeft tot het gepresenteerde handen, alsmede een verhoogde effectieve connectiviteit tussen deze regio en de rechter EBA. Dit resultaat suggereert dat de PMd kan compenseren door middel van een toegenomen connectiviteit met de posterieure regio’s.

Interferentie van corticale functie met behulp van cTBS bij gezonde controles
Het doel van het tweede deel van dit proefschrift was om de compenserende rol van deze eerder gevonden regio’s aan te tonen door actief te interfereren met de corticale activiteit. Hiervoor gebruikten we TMS. Onze hypothese was dat inhibtie van deze regio’s kan leiden tot meetbare veranderingen in de uitvoering van een (motor) taak. Hierrim konden we ook meer duidelijkheid krijgen over de rol van deze regio’s aan te tonen door actief te interfereren met de gepresenteerde handen in vergelijking met de controlegroep. Dit bevestigt dat er goede en op dezelfde manier de taak oplossen en dus dat de waargenomen cerebrale effecten niet kunnen worden verklaard door verschillen in de prestaties tussen de twee groepen. Ten tweede toonden LRRK2 dragers een verlaagde activiteit in de rechter nucleus caudatus tijdens toerenende rotatiehaken van de gepresenteerde handen in vergelijking met de controlegroep. Dit bevestigt dat er tijdens de premotore fase van LRRK2 parkinsonisme reeds cerebrale veranderingen gaande zijn. In tegenstelling tot de veranderingen in het putamen die beschreven zijn bij de idiopathische ziekte van Parkinson en bij de premotore dragers van een Parkin of PINK1 mutatie (Binkofski et al., 2007; Reetz et al., 2008), lijken de striatale veranderingen bij de premotore LRRK2 mutatiedragers te beginnen in de nucleus caudatus.

Ten derde lieten LRRK2 mutatiedragers in vergelijking met de controlegroep een toegenomen activiteit in de rechter PMd zien tijdens toerenende rotatiehaken van de gepresenteerde handen, alsmede een verhoogde effectieve connectiviteit tussen deze regio en de rechter EBA. Dit resultaat suggereert dat de PMd kan compenseren door middel van een toegenomen connectiviteit met de posterieure regio’s.

Interferentie van corticale functie met behulp van cTBS de ziekte van Parkinson
Hoofdstuk 6 heeft aangetoond dat het inderdaad mogelijk is om gedragsveranderingen en functionele veranderingen vast te leggen na inhibtie van een specifieke corticale regio met cTBS. We waren geïntrigeerd door een vorige fMRI studie, die
suggereerde dat de EBA een compenserende rol zou kunnen hebben bij patiënten met de ziekte van Parkinson tijdens het genereren van een motorisch plan (Helmičch et al., 2007), wat later door ons bevestigd werd in LRRK2 mutatiedragers (hoofdstuk 5). Met deze studies in gedachten hebben we een nieuw experiment opgezet waarin we dit gebied met behulp van cTBS geïnhibeerd hebben, om te testen of dit gebied inderdaad betrokken is bij compensatieprocessen (hoofdstuk 7). De gevolgen van deze interventie werden gemeten met een geëvalueerde taak: de verbeeldingstaak zoals gebruikt in hoofdstuk 5.

Hoofdstuk 7  gaf twee belangrijke resultaten. Ten eerste zorgde inhibitie van de EBA ervoor dat patiënten met de ziekte van Parkinson geen voordeel meer hadden als de eigen handpositie in dezelfde positie lag als de gepresenteerde hand. Dezelfde interventie had geen effect in de controlegroep, wat suggereert dat bij gezonde controles de EBA niet betrokken is bij de generatie van een motorisch plan, of dat andere gebieden compenseren voor de tijdelijke inhibitie van de EBA.

Een tweede bevinding was dat inhibitie van de PMd ervoor zorgde dat gezonde controles geen voordeel meer konden halen uit hun eigen handpositie in tegenstelling tot de groep Parkinson patiënten. We concludeerden dat Parkinson patiënten de PMd niet meer kunnen inzetten voor deze taak en dat deze dysfunction van de PMd gecompenseerd wordt door de EBA. Op basis van hoofdstuk 5 kon voorzichtig verondersteld worden dat deze rekrutering van meer posterieure gebieden als de EBA voor een geleidelijk falende PMd zich al in de premotorische fase afteken.

De functie van de premotore cortex in gezonde controles en de ziekte van Parkinson

Inhibitie van de linker PMd met cTBS voorkomt in gezonde proefpersonen maar niet in Parkinson patiënten dat de huidige lichaamshouding geïntegreerd wordt in een motorisch plan tijdens de verbeeldingstaak. Hieruit kunnen drie conclusies worden getrokken. Ten eerste is de linker PMd van Parkinsonpatiënten ongevoelig voor cTBS zoals gemeten met de verbeeldingstaak. Dit wijst op het feit dat de bekende overactiviteit van premotore gebieden bij de ziekte van Parkinson (Sabatini et al., 2000, Wu and Hallett, 2005, Eckert et al., 2006) waarschijnlijk berust op een dysfunction van deze gebieden in plaats van betrokkenheid bij compensatoire activiteit. Ten tweede is inhibitie van alleen de linker PMd bij gezonde proefpersonen voldoende om een verandering aan te brengen die ervoor zorgt dat de eigen lichaamshouding niet meer of minder wordt meegenomen in het motorisch plan, hetgeen een bevestiging is van de bekende hemisferale linkszijige dominantie van deze frontale regio tijdens de verbeeldingstaak (Hlustik et al., 2002, Haaland et al., 2004, de Lange et al., 2006). Ten derde waren gezonde proefpersonen niet in staat om andere circuits in te schakelen om de geïnhibeerde PMd te compenseren zoals gezien werd bij de patiënten met de ziekte van Parkinson. Dit wijst erop dat de EBA bij de ziekte van Parkinson waarschijnlijk inderdaad bij de aanwezigheid van compenserende mechanismen in het verband staat (Eckert et al., 2007), wat later door ons bevestigd werd in LRRK2 mutatiedragers (hoofdstuk 5).

Toekomstperspectieven

Functionele neuroimaging studies, zoals beschreven in de hoofdstukken 2 tot en met 5, hebben de eerste inzichten gegeven in de cerebrale structuren die betrokken zijn bij compensatietrategieën in de premotorische fase van de ziekte van Parkinson. De premotorische gebieden lijken een belangrijke rol te spelen in deze
van een relatief nieuw rTMS protocol (cTBS) (Huang et al., 2005) om corticale gebieden te inhiberen en de gedragssmatige gevolgen van deze interventie in kaart te brengen. Met deze methode hebben we laten zien dat de corticale gebieden die eerder gevonden zijn inderdaad betrokken lijken te zijn bij compensatie en bij het genereren van een motorisch plan in de ziekte van Parkinson. Toekomstig onderzoek moet laten zien of "het stimuleren van" deze compenserende gebieden met rTMS-protocollen leidt tot verbetering van de motore (en misschien zelfs cognitieve) symptomen van de ziekte van Parkinson. We kunnen hierbij een aantal problemen tegenkomen. Zo zijn de effecten van rTMS niet focaal (m.a.w. beperkt tot het vooraf bepaalde corticaal doelgebied), maar kunnen naar verder en/of dieper gelegen gebieden verspreiden. Ook op de plaats van stimulatie activeert TMS, zoals het momenteel gebruikt wordt, meerdere neurale populaties die verschillende neurotransmitters gebruiken en verschillende functies uitoefenen. Bovendien zijn de effecten van rTMS variabel en afhankelijk van de hersenactiviteit vooraf aan de stimulatie, de pathologische toestand van gestimuleerde netwerken en de gebruikte medicijnen (Huang et al., 2008). Deze kanttekeningen zijn echter niet allemaal negatief. De verspreiding van na-effecten naar diepere structuren kan ook gebruikt worden om juist deze diepere structuren als doelgebieden te laten dienen. Het grootste nadeel van TMS blijft de korte duur van de na-effecten. Herhaalde dagelijkse sessies kunnen leiden tot effecten die langer aanhouden (Baumer et al., 2003), maar de effecten blijven relatief klein. De vraag blijft dan ook of het wel realistisch is dat we therapeutische effecten kunnen bewerkstelligen met TMS. En moeten we de stimulatieparameters gebruiken die veilig blijken in gezonde controles of moeten deze veranderd worden voor patiënten? Net zoals bij medicatie-trials, zullen we moeten gaan testen welke stimulatieparameters het meest effectief blijken maar toch veilig zijn ("dose-finding").

Bovenop het potentiele “diagnostische” gebruik en de nieuwe inzichten in de pathofysiologie van de ziekte van Parkinson, wijzen onze resultaten (met enige voorzichtigheid) op nieuwe behandelingsopties voor de ziekte van Parkinson, bijvoorbeeld met TMS. In de hoofdstukken 6 en 7, hebben we gebruik gemaakt
CHAPTER 9

References


Dankwoord

List of publications

Curriculum Vitae

Dissertations of the Parkinson Centre
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Donders Graduate School
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Dankwoord


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Other:

Curriculum Vitae

Bart Franciscus Leonardus van Nuenen was born on June 1st 1978 in Tilburg, The Netherlands. After finishing Atheneum (St Odulphus Lyceum, Tilburg) in 1996 he started his medical studies first at the Catholic University Leuven in Belgium and one year later, in 1997, he continued his medical studies at the Radboud University Nijmegen in The Netherlands. In January 2004 he received his medical degree (MD) and started as a resident in Neurology at the Radboud University Nijmegen Medical Centre. In 2006 he started his PhD on cerebral reorganization in preclinical parkinsonism (promotor: prof. dr. B. Bloem), and performed a research fellowship (2006-2007) at the Christian-Albrechts-University Kiel in Germany, where he worked with prof. dr. H. Siebner and prof. dr. G. Deuschl. He investigated cortico-cortical connections in healthy subjects using multi-focal transcranial magnetic stimulation (TMS) and cerebral compensatory mechanisms in healthy subjects using multimodal imaging techniques (functional magnetic resonance imaging and TMS). In July 2011 he finished his residency in Neurology and continued working on his PhD at the Donders Centre for Cognitive Neuroimaging in Nijmegen. From January 2012 till June 2012 he is worked as a neurologist at the Slingeland Hospital Doetinchem. Currently he is working as a neurologist at the Catharina Hospital Eindhoven. Bart is married to Willemijn and father of Joep and Nina.
Dissertations of the Parkinson Centre Nijmegen (ParC)


Curriculum Vitae

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78. van Barneveld, D.C.P. B.M. (2012). Integration of exteroceptive and interoceptive cues in spatial localization. Radboud University Nijmegen, Nijmegen, the Netherlands.
86. Verhagen, L. (2012). How to grasp a ripe tomato. Utrecht University, Utrecht, the Netherlands.
Color Figures

Chapter 1

Figure 1.1
A. Photograph of a MRI scanner, the main magnetic field is 3 Tesla. Subjects lay supine in the scanner. Task stimuli are programmed on a computer in the control room, and presented onto the screen via a beamer. Subjects can respond in different ways (e.g. button press with hands or feet, or eye-movements with an eye-tracker). B. An image of the brain with areas of statistically significant areas when a healthy subject is asked to prepare to grasp and lift a weight.
Figure 1  Continued.  
Panel B (van Nuenen et al., 2009a): A two-dimensional drawing of the palm of the right hand was continuously presented in the centre of the visual field throughout the fMRI session. During the REST periods, the line drawing of the hand was continuously presented but without dots. Participants were instructed to remain still and fixate the hand with their eyes. During each block of TASK, participants performed three motor sequences. Each sequence consisted of three thumb-to-finger opposition movements. At the onset of each movement trial, the index, middle, ring or little finger was labeled with a red dot on a two-dimensional drawing of the palm of the right hand. The position and order of the red dot specified the motor sequence that had to be performed within a given trial. Participants sequentially tapped with the tip of their right thumb onto the tip of the indicated fingers after the instruction cue had disappeared from the screen. Participants were asked to move at a convenient speed and to perform the task as accurately as possible. There were 10 blocks of TASK and 10 blocks of REST.

Figure 2 Regional increases in task-related BOLD signal changes in non-manifesting carriers of a Parkin or PINK1 mutation. Statistical parametric maps.  
Sagittal, coronal, and axial slices highlighting those voxels in the left primary motor cortex, adjacent PMd and the right pre-SMA that showed a relative increase in BOLD signal during the sequential finger movements in the fast mutation carriers relative to the fast controls (upper row in A) and in the left caudal putamen and right pre-SMA that showed a relative increase in BOLD signal during the sequential finger movements in the slow mutation carriers relative to slow controls (lower row in A). All the statistical parametric maps are superimposed on to a T2-weighted structural MRI template provided by MRicro (http://www.sph.sc.edu/comd/orden/mricro.html). The voxels of the activation maps are colour-coded according to their Z-values. For illustrative purposes, the maps are thresholded at an uncorrected p-value of p < 0.01. Parameter estimates of task-related BOLD signal changes. The column plots (B) give the mean Beta-values (as estimated by the general linear model) for the task-related change in BOLD signal during the sequential finger movement task for each of the four groups (blue columns = mutation carriers; yellow columns = non-mutation carriers). The Beta values are given in arbitrary units (A.U.) and refer to the voxel showing a peak difference between mutation carriers and non-carriers. Error bars equal the 95% confidence interval of the mean.
Chapter 4

Figure 1. A. Experimental design. The fMRI session consisted of ten alternating periods without movements (REST) or sequential movements (TASK). Each block lasted for 24 s. There were 10 blocks of TASK and 10 blocks of REST. A two-dimensional drawing of the palm of the right hand was continuously presented in the centre of the visual field throughout the fMRI session. During the REST periods, the line drawing of the hand was continuously presented but without dots. Participants were instructed to remain still and fixate the hand with their eyes. During each block of TASK, participants performed three motor sequences. Each sequence consisted of three thumb-to-finger opposition movements. At the onset of each movement trial, the index, middle, ring or little finger was labelled with a red dot on a two-dimensional drawing of the palm of the right hand. The position and order of the red dot specified the motor sequence that had to be performed within a given trial. When the instruction cue disappeared from the screen, participants sequentially tapped with the tip of their right thumb onto the tip of the indicated fingers. They were asked to move at a convenient speed and to perform the task as accurately as possible.

Figure 1. B. Main effect of the motor task. The axial slices show the motor regions that showed a task-related increase in BOLD signal during the sequential finger movement task. The statistical parametric maps are superimposed on to a T2-weighted structural MRI template provided by MRicro (http://www.sph.sc.edu/comd/rorden/micro.html). The voxels of the activation maps are colour-coded according to their Z-values and thresholded at p<0.05 using the FWE method as implemented in SPM2.
Chapter 4

Figure 2  Regional increases in task-related BOLD signal changes in non-manifesting carriers of a Parkin or PINK1 mutation. 
(A). Statistical parametric maps. Sagittal, coronal, and axial slices highlighting those voxels in the pre-SMA and adjacent PMd that showed a relative increase in BOLD signal during the sequential finger movement task in mutation carriers relative to controls without a mutation. The statistical parametric maps are superimposed on to a T2-weighted structural MRI template provided by MRicro (http://www.sph.sc.edu/comd/rorden/ mricro.html). The voxels of the activation maps are colour-coded according to their Z-values. For illustrative purposes, the maps are thresholded at an uncorrected p-value of p<0.01.

Figure 3  Relative increases in task-related BOLD signal changes in non-manifesting carriers of a PINK1 mutation compared with non-manifesting carriers of a Parkin mutation. Axial slices showing voxels in dorsal frontoparietal cortex with significant increase in BOLD signal during the sequential finger movement task in PINK1 mutation carriers relative to Parkin mutation carriers. The statistical parametric maps are superimposed on to a T2-weighted structural MRI images provided by MRicro (http://www.sph.sc.edu/comd/rorden/micro.html). The voxels of the activation maps are colour-coded according to their Z-values. For illustrative purposes, the maps are thresholded at an uncorrected p-value of p<0.01.
Chapter 5

Figure 3 Cerebral effects.
A: Between-groups differences in imagery-related brain activity. Anatomical distribution of voxels that showed a differential change in BOLD signal as a function of stimulus rotation (i.e. imagery-related) between non-mutation carriers (NMC) and non-manifesting LRRK2 mutation carriers (MC). On the left, axial slice showing reduced imagery-related BOLD signal in the caudate nuclei; On the right, axial slice showing increased imagery-related BOLD signal in the dorsal premotor cortex (PMd). Statistical parametric maps (SPMs) of imagery-related between-groups differences (Z-scores, in red-yellow) superimposed onto a T2-weighted structural MRI template (in grey). For illustrative purposes, SPMs are thresholded at an uncorrected p value of p < 0.01 with a cluster extent threshold of > 50. B: Between-groups differences in imagery-related effective connectivity. The coronal slice on the right describes the anatomical distribution of voxels in the extrastriate body area (EBA) with differential increase in imagery-related coupling with the right PMd (left axial slice) between non-mutation carriers and non-manifesting LRRK2 mutation carriers. The SPM(Z) of between-groups differences in PMd-EBA imagery-related connectivity (in red-yellow) is superimposed onto a T2-weighted structural MRI template (in grey). C: Between-groups differences in the relation between imagery-related activity in the right caudate and PMd-EBA connectivity. Scatterplot of parameter estimates (β-values) of imagery-related activity in the right caudate (x = 18, y = 2, z = 18) against coupling strength between the right PMd (x = 34, y = -12, z = 46) and the right EBA (x = 40, y = -78, z = 4) for non-mutation carriers (left panel) and for non-manifesting LRRK2 mutation carriers (right panel).

Chapter 6

Figure 1 Experimental design.
(A) Time line of the experimental procedures. See methods section for further details. TMS/MEP = Measurements of motor evoked potentials (MEP) with single-pulse transcranial magnetic stimulation (TMS) of left primary motor hand area. cTBS = continuous theta burst stimulation. fMRI = functional magnetic resonance imaging. (B) Visually guided grip-and-lift force task. During fMRI, subjects were presented with a S1 pre-cue (red color) and a S2 go-cue (green color) with a variable delay between S1 and S2. The cues were projected on the screen for 1 s, thereafter an orange or grey cross was projected during a jittered period of 2 s to 8 s. The shape of the stimulus indicated the weight to be lifted. A circle or a square predicted a light (100g) or a heavy (250g) weight. In 75% of the trials the preparatory S1-cue correctly predicted the S2 cue. Depending on the combination of S1 and S2 cues, there were two trial types with correct pre-cue (HH = heavy-heavy and LL = light-light) and two trial types with incorrect pre-cue (LH = light-heavy and HL = heavy-light).
Chapter 6

Figure 6  Regional increases in BOLD signal during preparation (S1-S2 interval). (A) Main effect of preparation regardless of the weight indicated by the S1 pre-cue. The sagittal, coronal and axial slices show the regions that showed a increase in BOLD signal during the preparation of a lift (heavy and light). (B) Relative increases in BOLD signal during the preparation for lifting a heavy weight relative to preparing for lifting a light weight. The statistical parametric maps are based on the fMRI data recorded after sham cTBS\(_{30\%}\) of left PMd.

Figure 7  Linear relationship between regional activation during motor preparation and the relative undershoot in maximal grip force in trails where an incorrect S1 pre-cue announced a light weight after (A) sham cTBS\(_{30\%}\) or (B) real cTBS\(_{80\%}\) of left PMd. (A) In the control session without effective cTBS, preparatory activity during the S1-S2 period predicted the individual undershoot in maximal grip force. The higher the preparatory activity in the left PMd, the larger was the undershoot in trials with an incorrect S1 pre-cue indicating a light weight. (B) This linear relationship was abolished after real cTBS\(_{80\%}\) of left PMd. The left panels show axial slices of the statistical parametric map for the linear relationship between preparatory activity and force undershoot. The corresponding scatter plots for the peak voxel in left PMd are presented on the right (x, y, z = -30, -3, 54). The parameter estimates of preparatory BOLD signal changes are plotted along the y-axis. The maximal grip force ratios (LH / LL trials) are displayed along the x-axis. The grey color marks the area with negative LH/HH force ratio (i.e., undershoot). The regression line gives the estimated linear relation.
Chapter 6

Figure 8  Relationship between cTBS-induced change in force undershoot and weight-specific preparatory activity in left rostral SMA. Subjects in whom fMRI revealed a relative increase in preparatory S1-S2 activity for light lifts (relative to heavy lifts) after real cTBS\textsubscript{80\%}, showed no or little change in grip force undershoot (LH / HH ratio) after real cTBS\textsubscript{80\%}. Conversely, real cTBS\textsubscript{80\%} induced a clear reduction in grip force undershoot in those subjects who showed an increase in preparatory S1-S2 activity for light lifts (relative to heavy lifts) after real cTBS\textsubscript{80\%}. The axial slice (upper panel) illustrates the cluster in left rostral SMA showing a linear relation between cTBS-induced change in force undershoot and weight-specific preparatory activity. The corresponding scatter plot for the peak voxel in left rostral SMA is illustrated in the lower panel (x, y, z = -6, 18, 54). The regression line gives the estimated linear relation.