Electrophysiological Biomarkers in Genetic Movement Disorders

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PhD Thesis

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I, Susanne A. Schneider, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

ABSTRACT

Background: Neurodegenerative diseases are diseases of the nervous system with progressive course leading to death. Treatment remains symptomatic. Development of neuroprotective agents has been hampered for various reasons. This includes the inability of making the diagnosis accurately early in the course and the lack of reliable disease progression markers which could be used in future treatment trials.

Transcranial magnetic stimulation (TMS) is a non-invasive and pain-free method for assessment of brain function.

Methods: Here we evaluated TMS and its potential of serving as reliable biomarker for neurodegenerative diseases with genetic cause.

After clinical delineation of our patient cohorts with Huntington's chorea and young-onset Parkin-related Parkinsonism, we enrolled both patients as well as asymptomatic/ presymptomatic gene-carriers. Patients, carriers and age-matched healthy controls were studied using TMS to establish an electrophysiological footprint of these conditions.

Results: We found abnormalities in electrophysiological parameters which were present in manifesting patients and/or non-manifesting gene mutation carriers.

In HD, both presymptomatic and early manifest patients had increased resting and active motor cortex thresholds. Short afferent inhibition (SAI), a measure of sensory-motor integration, was reduced in manifesting patients only. SAI changes were inversely correlated with clinical parameters like predicted years to onset and UHDRS motor score.

Abnormalities in Parkin patients included prolonged central motor conduction time (CMCT), while thresholds and cortical inhibitory activity were normal. Asymptomatic carriers had increased motor thresholds and abnormal inhibitory measures (SICI recruitment) while CMCT was normal.

Conclusion: We conclude that TMS may be a potential biomarker for neurodegenerative genetic diseases 1) to detect changes early in the disease course and to monitor disease progression; 2) to help differentiating between clinically similar diseases on the basis of certain electrophysiological patterns; and 3) to give insight into underlying mechanisms of the disorders studied. Our findings suggest the potential for future research.

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A) Preface

I. Acknowledgements

I would like to cordially thank Professor Kailash Bhatia and Professor John Rothwell for their invaluable guidance and support throughout my projects. Similarly, I am most grateful to Professor Niall Quinn and Professor John Hardy for their support in many ways. I would also like to thank my co-workers, with whom much of this work was performed, in particular Dr Penny Talleli, Dr Sven Schippling and Dr Coro Paisan-Ruiz. Of course there are many other researchers who I collaborated with during my PhD period who I am grateful to – and this is a long list – however in order not to forget anyone I do not even dare naming them individually.

I am very grateful to the Brain Research Trust and Lady Astor for the generous financial support without which my stay in London would not have been possible.

Furthermore, I would like to thank the patients and their families who gave their precious time and support to this project, without whose generosity this work would not have been possible.

Finally, I would like to thank my family and friends who supported me all along.

II. Statement of Participation in Studies Described

The initial concept for the thesis was generated by Kailash Bhatia and John Rothwell and was then developed by myself. Drs Penny Talleli and Sven Schippling played a key role in the design and practical execution of the studies. I participated in all the electophysiological studies described here. Patient ascertainment was conducted by myself. Analysis and interpretation of data was conducted by myself, my co-workers, Kailash Bhatia and John Rothwell.

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IV. Abbreviations

UHDRS - Unified Huntington's Disease Rating Scale. A scale used to measure the severity of Huntington's disease. A higher score denotes greater disability

UPDRS - Unified Parkinson's Disease Rating Scale. A scale used to measure the severity of Parkinson's disease. A higher score denotes greater disability

V. Thesis Overview

Chapter 1 will introduce the topic of neurodegenerative disease. The concept of biomarkers as a measure of disease progression and their importance towards identification of neuroprotective agents will be discussed. The terms 'mode of inheritance', 'penetrance', 'manifesting' and 'non-manifesting carriers' and 'pre-symptomatic gene carriers' are introduced. The idea (and hypotheses) of using transcranial magnetic stimulation as a possible biomarker is formulated.

Chapter 2 provides a review of the diseases studied here, Huntington's disease and Parkin-related Parkinsonism.

Chapter 3 entails the clinical experiments. A clinical delineation of these conditions, their phenocopies and related syndromes are discussed.

Chapter 4 introduces the technique of transcranial magnetic stimulation, the method used for the experiments described in this thesis.

Chapter 5 presents a literature review on the use of transcranial magnetic stimulation in the patient groups of interest and related disorders; in other words, a literature review on TMS in genetic movement disorders.

Chapter 6 describes the methods of the performed experiments: subject ascertainment, electrophysiological parameters and methods of data analysis.

Chapter 7 presents the results of the TMS experiment in Huntington's disease.

Chapter 8 presents the results of the TMS experiment in Parkin disease.

Chapter 9 delivers a discussion of the findings in view of the hypothesis of the thesis and in context of findings from other research groups.

Chapter 10 delivers a conclusion. Suggestions are being made in which direction future work could go.

B) Thesis

Chapter 1

I. Neurodegenerative Disease and the concept of biomarkers

The term "neurodegeneration" originates from the Greek word νέυρο-, néuro-, "nerval" and the Latin word dēgenerāre, "to decline" or "to worsen" and refers to conditions characterized by progressive dysfunction of the nervous system. Figure 1.1 demonstrates the time course of neurodegernative diseases.

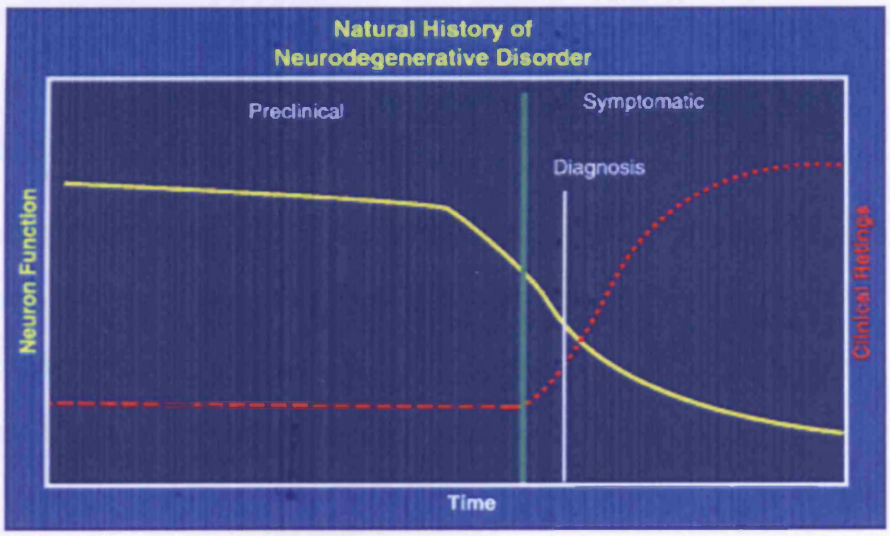

Figure 1.1. Natural history of a neurodegenerative disorder. Over time neuronal survival and neuronal function declines (disease onset, yellow line). Eventually, surviving cells cannot compensate for longer, clinical symptoms (red dotted line) become overt (green vertical line) and worsen in parallel to neuronal loss. A diagnosis may be made at a later stage (white vertical line).

Atrophy of the affected central or peripheral nervous system structures are often associated. According to the function of the neurons lost, the sympomatology can vary and may include movement disorders or/and dementia or other functional systems. Important examples of neurodegenerative diseases are Parkinson's disease and Alzheimer's disease, however, the list of conditions is long. Both hereditary and sporadic forms occur.

Treatment of neurodegenerative diseases is difficult and often remains unsatisfactory. To date, only symptomatic treatments are available. No proven treatments that delay the onset or prevent the progression of neurodegenerative diseases have yet been discovered and it is a major scientific and public health goal to identify neuroprotective agents.

The development of treatments has been hampered by three challenges: 1) the underlying mechanisms causing the progressive cell loss remain ill understood, 2) the lack of tools for accurate ante-mortem diagnosis and 3) the lack of reliable disease markers to monitor disease processes, especially in earliest disease stages.[1]

While elucidating disease mechanisms is not a prerequisite for the development of therapies, testing and implementing such therapies requires identification of appropriate patient populations, especially those individuals in the preclinical (asymptomatic) stages.[1] For this, antecedent disease biomarkers (i.e., markers of neuropathologic disease in the absence of any clinical symptoms) need to be identified and carefully characterized in order to test, challenge and validate any

possible therapies capable of preventing or delaying the onset of neuronal death and consequent clinical presentation in the appropriate populations.

Clinically useful biomarkers require certain characteristics. They should be precise, reliable and reproducible. Numerous potential biomarkers are currently being explored, challenged and evaluated.

Pathological markers which may indicate generalized processes or even those with more direct relevance to the specific disease (e.g. Lewy bodies in Parkinson's disease or Aß and tau in Alzheimer's disease) may not be clinically useful for diagnosis or monitoring during life, as ascertaining brain biopsies is obviously limited because of the invasive nature.

In inherited disorders, fragment of DNA sequences that causes disease or are associated with susceptibility to disease can serve as genetic biomarkers (genetic marker, e.g. trinuleotid repeat in Huntington's disease or mutations or rearrangements in Parkin-related parkinsonism). Genetic markers may help to confirm the diagnosis in a patient and in some conditions may permit predictive testing in the presymptomatic phase. Identification of genetic markers are also the gate to pathophysiological insights. However, genetic testing does not permit disease monitoring.

In this thesis the potential use of the electrophysiological technique, transcranial magnetic stimulation as a useful biomarker will be assessed and discussed in the following.

II. Introduction to Transcranial Magnetic Stimulation

Transcranial Magnetic Stimulation (TMS) is a non-invasive electrophysiological technique of stimulating cortical neurons which allows assessment of the central and peripheral nervous system. Since its development by Barker in 1985, a variety of TMS paradigms have been developed and intensively investigated. This includes measures of cortico-cortical connectivity, cortico-spinal connectivity, both excitatory and inhibitory mechanisms, and plasticity. Insights into correlations between TMS measures and underlying cellular mechanisms, namely which neurotransmitter may be involved, have been proposed based on pharmacological modifiability of TMS measures and previous neuropathological findings. Certain electrophysiological abnormalities or patterns may be indicative of distinct disorders. Furthermore, correlations between TMS abnormalities and clinical findings (e.g. severity of overall disease or individual symptoms) have been explored for numerous diseases.

Thus, in view of the painfree and non-invasive character and the correlation with functional systems and clinical findings TMS represents an interesting potential biomarker for neurodegenerative disease.

III. Mode of inheritance, penetrance and manifesting versus pre-symptomatic/ non-manifesting subjects

In Mendelian genetics two forms of inheritance can be distinguished. Autosomal dominant inheritance is characterized by alleles that express themselves at the expense of alternate alleles, thus a single heterozygous pathogenic mutation in one of the two alleles is sufficient to cause disease. By contrast recessive traits

are defined by the fact that expression of an allele is suppressed in the presence of a dominant allele and thus two mutations (either the same mutation (homozygous) or two different changes in the two alleles (compound heterozygous)) are needed for production of symptoms.

For autosomal dominant conditions, the genetic term of "penetrance" also becomes interesting in the context of the proposed studies and is defined as the proportion of individuals carrying a particular variation of a gene (an allele) that also express a particular trait (the phenotype). Full penetrance means that every individual carrying a gene mutation will develop symptoms eventually whereas reduced (or incomplete) penetrance refers to conditions where not every gene carrier develops symptoms or signs. Reduced penetrance probably results from a combination of genetic, environmental, and lifestyle factors, many of which are unknown. An example for a movement disorder with high, basically complete, penetrance is Huntington's disease. On the other hand, penetrance is relatively low for DYT1 dystonia. Only about 30% of patients carrying a mutation will develop symptoms. Based on an age-dependency of manifestation of symptoms, one can be reasonably confident that so-called "non-manifesting DYT1 gene carriers" over the age of thirty will not manifest symptoms in the future and thus can be considered as truly different from manifesting gene carriers.[2,3] Such non-manifesting gene mutation carriers are of particular interest to researchers as they allow studying consequences of the mutated gene other than clinical symptoms.

With respect to recessive disorders, in recent years, several monogenic causes of disease have been identified. This includes parkinsonism where mutations can be detected in about 3% of patients, but the proportion can be as high as 77% in groups of patients selected for age at onset, positive family history[4], and ethnic

origin.[5,6] Similarly to non-manifesting gene carriers of dominant disorders, hetereozygous carriers of an autosomal recessive disease are of particular interest to researchers. These subjects carry one mutation, which – as defined by the recessive inheritance – is not enough to produce a full blown phenotype. However, there has been increasing debate whether and how the gene mutation may show its hand and whether carriers may be predisposed to developing clinical symptoms later in life, for example idiopathic Parkinson's disease as it has been reported for individual cases with parkin- or PINK1-related parkinsonism.[7]

Studying non-manifesting individuals may give insight into the pathophysiology of the disorder. Studying these individuals in addition to patients may furthermore allow challenging the method (in our case transcranial magnetic stimulation) regarding its potential of monitoring disease processes, even in the pre-clinical phase. We have thus chosen to study clinically affected patients as well as non-affected gene mutation carriers. We have chosen Huntington's disease as an example of autosomal dominant inheritance and Parkin-related parkinsonism as recessive disorder.

IV. Aims and Hypotheses

We had two main aims. Our first aim was to clinically delineate genetic neurodegenerative diseases. Because lumping of clinically similar conditions may hamper their workup (identification of genetic causes and selection of populations for future therapeutic trials), delineation of these disorders is an important first step.

Our second aim was to use electrophysiological techniques to probe the function of the motor system in patients with genetic movement disorders. We applied the techniques in patients with clinical disease as well as those without clinical symptoms, for the reasons outlined above, that is to evaluate the potential usefulness of TMS as a biomarker.

We set out the following main hypotheses for our experiments:

1. That electrophysiological abnormalities present in the studied patients may give insight into the pathophysiology of disease.
2. That certain TMS parameters may be useful markers to differentiate between disorders, thus patients with distinct genetic disorders from those without (Mendelian) genetic mutations with a similar clinical presentation (sporadic disease) or those with a distinct other genetic disorder.
3. That non-manifesting/ premanifesting gene carriers show electrophysiological abnormalities which reflect subtle pre-clinical abnormalities and that these affect similar systems to those seen in manifesting gene carriers
4. That TMS may be useful to measure progression of neurodegenerative diseases.

Chapter 2 Clinical Delineation of Genetic Movement Disorders

I. Huntington's disease – an example of autosomal dominant inheritance

Huntington's disease (HD), a neurodegenerative autosomal dominant disorder due to mutation of the Huntingtin gene (IT15) on chromosome 4[8] is the most important cause of genetic chorea. More precisely, HD is due to prolongation of a physiological polyglutamine stretch which ranges from 27 to 35 in healthy. Penetrance is incomplete between 36 and 39 repeats and individuals may or may not develop disease. Ranges of 40 or more repeats eventually cause HD and the longer the stretch the more severe the clinical phenotype. Disease onset is inversely related to the number of repeats. [9] [10]

The prevalence of HD has been found high in regions of Venezuela and Scotland and relatively low for Japan, Finland and Norway. [11] [12] In Europe and North America the prevalence is about 4-8 per 100 000.

Onset of classic HD is around age 40 with a combination of personality changes, generalized chorea, and cognitive decline. However, when affecting children or adolescents, HD tends to present as akinetic rigid variant (Westphal form) rather than with chorea. This young-onset variant is more commonly inherited from the father due to meiotic instability with an increased risk of expansion. Other features in both adult and young onset forms include eye movement abnormalities (impersistence of gaze, difficulty initiating saccades), dysarthria, dysphagia, pyramidal signs and ataxia resulting in walking with imbalance and reduced postural stability. Dystonia, myoclonus, tics and tremor are also

compatible with a diagnosis of HD. Progression is inexorable with death after 15-20 years.

Brain imaging reveals progressive atrophy of the caudate nuclei, present years before onset of symptoms.

The diagnosis is based on genetic testing. Positive testing has major implications on the entire family and genetic counselling should be offered to explain risk of inheriting the disease (50%) and penetrance (100% when the polyglutamine stretch exceeds 40 repeats).[9]

Therapy remains symptomatic. Tetrabenazine reduces chorea through presynaptic dopamine depletion and mild D2-receptor blockage. Typical and atypical neuroleptics also reduce chorea. However, chorea is usually more bothersome for relatives and carers than for patients. Furthermore, if medication is excessive, motor abilities may markedly deteriorate as chorea becomes replaced by disabling hypokinesia and bradykinesia, hence, primary aim should be to lessen chorea rather than fully suppress it.

Depression can be treated with classical antidepressants. Deep brain stimulation of the globus pallidus internus has been reported to bring temporary benefit in single case reports but can not stop neurodegeneration.[13] Multiple drug trials are currently being carried out hoping to find neuroprotective agents.

The exact underlying mechanisms of HD are not fully understood. Aggregation of mutant protein fragments, interference with transcription factors and gene expression, abnormal levels of nerve growth factors and calcium, mitochondrial dysfunction, reduction of synaptic transmission resulting in interference of signaling pathways seem to play a role. [14] [10] [15]

II. Parkin-related Parkinson's disease - an example of autosomal recessive disease

Idiopathic Parkinson's disease is characterized by the triad of bradykinesia (fatiguing of repetitive movements), tremor and rigidity. Typically, age of onset is in late adulthood, however young-onset (onset before age 40) and juvenile-onset forms occur. Whereas classic Parkinson's disease is considered idiopathic, twenty percent of patients with young-onset Parkinson's disease have a positive family history in that at least one first- or second-degree relative in the same or antecedent generations was also affected by parkinsonism. [16]

In recent years, several loci of parkinsonism with dominant (e.g. PARK8 related to leucine-rich repeat kinase 2(LRRK2)) and recessive (e.g. PARK2 related to parkin[4] or PARK6 related to PTEN-induced putative kinase 1 (PINK1)[7,17]) inheritance have been discovered. Of these, parkin-related parkinsonism (OMIM 602544) is the most common cause of autosomal recessive "young-onset Parkinson's disease" – it accounts for approximately half of the familial cases with disease onset before age 40 and for 75% in those with onset age 20 years or younger (juvenile PD). On the other hand, parkin mutations are unlikely (<5%) in patients with later onset (after age 30 years).[4] The clinical phenotype is dominated by levodopa-responsive parkinsonism with a benign course. Generally, the younger the age at onset the slower the evolution. [18,19,20] Dystonia usually affecting the lower limbs may be a presenting sign in up to 40%.[4] Exercise-induced dykinesia/dystonia as presenting sign of parkin-related parkinsonism has also been described.[21] Pyramidal features in the form of brisk reflexes occur in about half of the patients. [22] However, both dystonia at onset

and brisk reflexes may not be a consequence of the presence of the parkin mutation, but correlate better with the early onset age.[20]

Overall, no clear clinical signs to distinguish idiopathic Parkinson's disease and parkin-related parkinsonism have been identified, although one study suggested that sense of smell may be preserved in parkin.[23] However, neuropathologically, parkin is distinct from idiopathic Parkinson's disease where Lewy bodies are a hallmark feature. In parkin Lewy bodes are absent or scarce[24-26], although this remains matter of debate.[27,28]

Parkin mutations associated with PD affect all exons, and include point mutations, small insertions/deletions and much larger deletions, and exon duplications and triplications.[29,30] Most parkin[31] patients carry two different mutations (compound heterozygotes). A genotype-phenotype analysis of 146 patients with and 250 patients without parkin mutations, suggested disease severity (as measured by the United Parkinson's Disease Rating Scale motor score) may be greater in carriers of at least one missense mutation compared to those carrying two truncating mutations.[20] The localization of the mutations also played a role; missense mutations in functional domains of parkin resulted in earlier onset. Patients with a single heterozygous mutation had significantly later and more asymmetrical onset and more frequent levodopa-induced fluctuations and dystonia than patients with two mutations.[20]

The role of a single parkin mutation remains controversial and it is being debated whether asymptomatic parkin heterozygotes (carriers) are predisposed to developing (late-onset) parkinsonism. Cohort studies revealed unequivocal results.[32,33] However, it has been shown that carriers can have mild extrapyramidal signs and perhaps a susceptibility to behavioral disorder as well

as nigrostriatal dysfunction on functional imaging[34,35] and discrete abnormalities on voxel-based morphometry[36].

Chapter 3 Clinical Experiments -- Clinical delineation of Huntington's disease and Parkin-related young-onset Parkinson's disease

During patient ascertainment for this study, we have encountered patients (and families) with phenotypes indistinguishable from or similar to Huntington's disease and Parkin-related parkinsonism. However, these patients turned out to not carry the relevant gene mutation. This prompted a detailed work-up with genetic analysis of such cases as well as a review paper. Some of these data will be presented in the following.

I. Clinical delineation of Huntington's disease

1. Phenotypic homogeneity of the Huntington's disease-like presentation in a SCA17 family

The family of a 50-year-old Caucasian woman[37] carried a diagnosis of Huntington's disease, based on clinical details of affected family members and results of available chromosome 4 haplotype data in the early 1990's. At that time, she was unaffected and was counseled as having a low risk of disease manifestation based on the linkage data.

She developed clinical features compatible with HD, including generalized chorea, cognitive and behavioral changes, and eye movement abnormalities at age 44. Genetic testing for an *IT15*/HD expansion carried out by us was negative. The syndrome affected five members of the family suggestive of autosomal dominant inheritance. Onset was around age 40 (range 37-45).

Because of additional clinical features other conditions including DRPLA were considered. DRPLA can present with a combination of chorea, myoclonus, cognitive impairment, and cerebellar ataxia. However, repeat expansions in the

atrophin-1 gene were excluded. Choreo-acanthocytosis was excluded by peripheral blood smears. Also, inheritance of classic choreo-acanthocytosis is usually autosomal recessive. Among the HD-like (HDL) syndromes, HDL1, a familial prion disease due to mutation in the *PRNP* gene, is a more progressive disease with an average duration of 1 to 5 years.[38] HDL2, caused by dominant mutations in the *junctophilin*-3 gene, has not been reported in Caucasians.[39] HDL3 is an autosomal-recessive condition originally described in a Saudi-Arabian family.[40] Finally, HDL 4 or SCA17 can present with a clinical picture indistinguishable from classical Huntington's disease (also see section Chapter 3.I.2d). This has been documented in both heterozygous and homozygous *TBP* mutation carriers, although most of these patients also had some cerebellar signs.[41, 42, 43, 44]

In view of the cerebellar involvement, HDL4/SCA17 was considered likely and mutation analysis of the *TBP* gene eventually revealed a pathological expansion of 46 CAG/CAA repeats A diagnosis of SCA17 was made.

With hindsight, some features in our patient were atypical for HD including a myoclonic component of movements (although cortical myoclonus has been observed in HD). Second, her gait had an additional ataxic component. However, the gait disturbance in HD is usually classified as a frontal gait disorder with mixed hypokinetic-rigid and ataxic features, in which the postural imbalance, chorea and motor recklessness contribute to frequent falls. Thus, the gait in our patient was not incompatible with HD. Third, and most importantly, she showed mild upper limb ataxia, and cerebellar atrophy was present on neuroimaging. Although cerebellar involvement has been reported in HD, this is very rare in

adult onset cases and usually consists of mild cerebellar ataxia in early disease stages.

SCA17 HDL presentation has to date been observed only sporadically or in solitary individuals within a family. HDL phenotypic homogeneity in SCA17 has not been described. Our family is an example of that SCA17 can present with a HDL syndrome in multiple family members.

2. What to consider in a patient with a negative HD gene test? - The Huntington's disease like (HDL) disorders

Since identification of the HD gene in 1993 [8], it has been recognized that what appears to be HD phenotypically, is genetically heterogeneous and a small proportion of patients with a clinical syndrome of HD do not have the HD-causing trinucleotide repeat expansion in the *IT15* gene. For example, in a report of 618 patients [45], only 93% of those with a classical clinical phenotype of HD were found to have the HD mutation, thus providing evidence for the existence of other genetic disorders which are referred to as "Huntington's disease-like" (HDL) syndromes. A number of unrelated genes have recently been identified.[38-41] Thus, clinicians have to consider an increasing range of differential diagnoses when confronted with a patient with slowly progressive, adult-onset chorea and a positive family history. In view of this growing list of recognized disorders, we have reviewed the commonest genetic causes of chorea with focus on the spectrum of HD-like disorders (Review in Nature Clin Practice[46]). We concentrated on the autosomal dominant conditions. However, because about 8% of HD patients present without apparent family history, chorea with other modes of inheritance should also be considered.

a) HDL1

HDL1 is an autosomal dominant progressive adult-onset neurodegenerative disorder due to insertions of 192bp-nucleotides and 168bp-nucleotides encoding extra octapeptide repeats in the region of the P*rion protein gene (PRNP)* on chromosome 20p12.[38,47,48]. The clinical picture may be similar to HD with abnormal involuntary movements, difficulty in coordination, dementia, personality changes and psychiatric symptoms. [49] Seizures have been also described [38]. Mean age at onset is 20-45 years. Atrophy of the basal ganglia, the frontal and temporal lobes [38] and the cerebellum, with kuru and multicentric plaques labeled with anti-prion antibodies was demonstrated on neuropathological exam. [47,49] Despite the clinical suggestion of spongiform encephalopathy, spongiosis was not prominent. The normal form of the encoded prion protein (PrP^C) is attached to the cellular membranes through a glycophosphatidylinositol anchor and has – among others- a copper binding site. The normal function of the protein, however, is ill understood. [50] The conformational conversion and transformation of the cellular isoform to the pathogenic protein is believed to play a main role in pathogenesis. It has been suggested that the site of this formation and subsequent accumulation affecting different brain regions may contribute to the broad phenotype and existence of pathologically distinct prion disease entities. [51] Overall this seems to be a rare genetic cause of HD phenocopies[43,52 53].

b) HDL2

HDL 2 appears to be responsible for about 2% of patients without the *IT15* mutation. [43,45] The frequency is, however, higher in black South Africans where HDL2 contributes significantly to the HD phenotype. [54-56] To assess this further, Krause et al. [56] genetically tested 149 South African patients with a HD phenotype.

Whilst 84% (78/93) of white patients had the *IT15* expansion, only 36% (18/50) of black patients and 50% (3/6) of mixed-ancestry patients were found to have the HD-causing mutation. However, 24% (12/50) of black patients and 50% (3/6) of mixed-ancestry patients had HDL2-causing expansions. It has been suggested that the disorder is due to a founder effect originating in Africa between 300 and 2000 years ago. [56] North American and Mexican HDL2 families with African origins have been described. [57] With the exception of one Brazilian family of Spanish/Portuguese ancestry [58], HDL2 has not been reported in Caucasian or Japanese.[52,53,55,57]

Onset is in the third-fourth decades with a clinical picture resembling classic adult-onset HD. Similarities with the juvenile-onset variant have also been described (pedigree W), however, with the absence of seizures and often normal eye movements. [39]

Pathological examination showed a picture indistinguishable from classic HD [54,59]. The disorder is caused by a CTG/CAG expansion on chromosome 16q24.3 in the *Junctophilin-3* gene [60] with an inverse correlation between age of onset and repeat length. The function of junctophilin remains unknown but a role in junctional membrane structures and in the regulation of calcium has been suggested. [61]. In the normal population, the repeat length ranges from 6 to 27 CTG/CAG triplets [61]. Pathological repeat expansions range from 43 to 57 triplets, with length instability in maternal transmission. [54]. To date, the impact of alleles with from 36 to 39 triplet repeats is uncertain. [54] Pathogenicity may be related to the presence of mRNA inclusions [62].

In about 10% of cases with HDL2, acanthocytes can be detected in the peripheral blood smear. [61,63]

c) HDL3

Al-Tahan et al. [64] and Kambouris et al. [40] reported an autosomal recessive variant of HD from Saudi Arabia presenting with early-onset mental deterioration, dysarthria, dystonia, pyramidal signs, ataxia, and gait impairment. Onset age was 3-4 years. Progressive atrophy of the caudates bilaterally and the frontal cortex was demonstrated by brain imaging. The condition, named HDL3, was mapped to chromosome 4p15.3 [40], however, weakness of the evidence has been suggested [65]. Although this condition has been named HDL3, it does not fit into the group of HDL syndromes with respect the age of onset and the pattern of inheritance.

d) HDL4 / Spinocerebellar ataxia 17

HDL4 has now been identified as spinocerebellar ataxia (SCA) 17 and is an autosomal dominant triplet repeat disorder due to mutation of the *TBP* gene, encoding for the TATA box-binding protein (TBP), an important general transcription initiation factor, on chromosome 6q27.[66] Normal CAA/CAG repeat stretches range from 25 to 42 in Caucasians, with larger repeats considered pathological. Reduced penetrance has been reported for alleles with 43-48 CAG repeats.[67] Onset age is between 19 and 48 years with rare childhood onset.[68] Similar to HD, there is an inverse correlation between the age of onset and the number of repeats. Although cerebellar ataxia is the most common feature (94%), the phenotype is markedly heterogeneous, and extrapyramidal (73%), pyramidal (37%), epilepsy (22%), dementia (76%), or psychiatric disturbances (27%) may be prominent. [69] A clinical picture indistinguishable from classical HD has been reported in both heterozygous and homozygous mutation carriers.[41,70] Although within most families an HD-like presentation is observed only sporadically, a homogeneous HDL phenotype in all members of a SCA17

family has recently been described by us (see section Chapter 3 I.1).[37] The broad spectrum of clinical manifestations correlates with the neuropathological findings of cerebellar pathology, involvement of the cerebral neocortex, basal ganglia, and hippocampus.[71] Intranuclear neuronal inclusion bodies with immunoreactivity to anti-TBP and anti-polyglutamine widely distributed in the grey matter have been described. [71]

Cerebellar and cortical atrophy are demonstrated on brain MRI. [37] Neuroimaging studies using DAT- and IBZM-SPECT, and PET revealed reduced activity of dopamine transporters, reduced glucose metabolism in the striatum and mildly reduced dopamine D2 receptor-binding capacity. [72]. In patients with the more common ataxic phenotype voxel-based morphometry showed degeneration of the grey matter in the cerebellum, occipito-parietal cortical areas and the basal ganglia, reflecting and associated with the cerebellar, pyramidal and extrapyramidal signs. [73] Similarly, ataxic patients have been studied electrophysiologically. [74] EMG, nerve conduction studies, visual and brainstem auditory evoked potentials, and transcranial magnetic stimulation-induced motor evoked potentials appeared normal in all. However, somatosensory evoked potential abnormalities consisting mainly of P14 and P31 wave absence and a prolonged central motor conduction time were noted.

e) A summary of HDL disorders

To sift through the HDL disorders, one must pay attention to certain clinical features. Prominent myoclonus may indicate HDL1 or DRPLA. On the other hand, if cerebellar signs are marked, or cerebellar atrophy is demonstrated on neuroimaging, the SCAs and DRPLA come into consideration. In the latter condition, high-intensity signals of the cerebral white matter, the basal ganglia

and brain stem on T2-weighted MRI, in addition to atrophy of the brainstem and cerebellum, may be present. However, this is predominantly observed in late-onset adult DRPLA patients with a long disease duration and only rarely observed in juvenile patients with a short disease history. PKAN and neuroferritinopathy have characteristic MRI imaging, and additionally chorea is rarely an isolated movement disorder in these conditions in which dystonia and other features may be dominant. Prominent orolingual involvements with dystonic tongue protrusion are characteristic of PKAN and chorea-acanthocytosis [75] and these disorders also differ from classic HD in their pattern of inheritance.

Treatment for the conditions remains symptomatic. Tetrabenazine or dopamine receptor-blocking agents can alleviate the chorea. More disabling for the patient however, often are the psychiatric features and mood disturbances, in which case antidepressants may be indicated. Genetic counselling is facilitated by making a molecular diagnosis, which is increasingly possible, although may still be challenging. Social services and ancillary agencies support, as well as occupational, speech and physiotherapy are important components of treatment, and should not be neglected.

3. Huntington's disease phenocopies are clinically and genetically heterogeneous

In a further step, we wished to ascertain the prevalence of HDL disorders among patients who are negative for HD mutations. We thus screened DNA samples of 285 patients with syndromes consistent with HD, who were HD expansion-negative, for mutations in *PRNP, JPH3, TBP, DRPLA, SCA1, SCA2, SCA3, FTL* and *FRDA*.

Table 3.1 Clinical characteristics of subjects successfully diagnosed by genetic analysis in this 285-patient HD phenocopy cohort (from Wild et al[76])

We[76] identified five cases of HDL4, one of HDL1 and one of HDL2. One patient had Friedreich's ataxia. There were no cases of DRPLA, SCA1, SCA2, SCA3 or neuroferritinopathy. A summary of the clinical characteristics of subjects successfully diagnosed by genetic analysis in this 285-patient HD phenocopy cohort is given in table 3.1.

Our work led to the conclusion that among patients with a HD like condition a definitive genetic diagnosis is currently possible in only a minority of cases.

II. Clinical delineation of young-onset parkinsonism and related disorders

As mentioned above the onset of parkin-related parkinsonism may be with exercise-induced paroxysmal dyskinesias. These are characterized by involuntary movements triggered by prolonged exercise. Symptomatic forms may be due to lesions in the basal ganglia. Primary cases may be sporadic or inherited. Underlying gene mutations have not been identified.

1. GLUT1 mutations are a cause of paroxysmal exercise-induced dyskinesias

During the PhD period, in international collaboration, we have been able to identify the underlying gene causing paroxysmal-exercise induced dyskineias.

We identified mutations in the GLUT1 gene.[30,77] A cation leak resulting in reduced glucose transport into erythrocytes, as demonstrated by electrophysiological and transport assays, may play a pathophysiological role in some of the cases who have hemolysis or echinocytosis.

2. Parkinsonism-Dystonia – characterization of PLA2G6 as a causative locus

In patients with recessive dystonia-parkinsonism, the differential diagnosis is complex. In addition to the parkinsonism loci *PARK2*[31], *PARK6*[78] (chromosome 1p, OMIM 608309), *PARK7*[79] (chromosome 1p, OMIM 602533) and the dopa-responsive dystonias, but also pantothenate kinase-associated neurodegeneration (PKAN, also called Hallervorden Spatz disease or Neurodegeneration with Brain iron Accumulation (NBIA) type 1) caused by mutations in *PANK2* on chromosome 20 (OMIM 234200)[80], Kufor-Rakeb syndrome caused by mutations in *ATP13A2*[81] on chromosome 1p (OMIM 606693) and *DYT16* linked to mutations in PRKRA on chromosome 2[82] may present with parkinsonism-dystonia, some of these syndromes complicated by pyramidal or other features.

In order to find the causes of parkinsonism dystonia in consanguineous families and to better group them for clinical characterization and to identify any families which do not have mutations in the known loci, we have embarked on a systematic screening using whole genome genotyping methods[82] to identify which families show homozygosity at the different loci and then sequenced the family members to find the homozygous gene mutations. We identified homozygosity on chromosome 22 and, subsequently *PLA2G6* mutations in two

families. These individuals did not fit the previously described phenotype of this syndrome, which is of Infantile Neuroaxonal Dystrophy (INAD) which is clinically characterized by mental retardation, early cerebellar degeneration pyramidal signs and visual disturbances.[83] In contrast, our two unrelated families with a yet unrecognized phenotype of PLA2G6 gene mutations that is adult-onset complicated parkinsonism without brain accumulation on brain MRI.

Age of onset was in early adulthood, at age 26 and 18 in our index cases, however there was intrafamilial heterogeneity with one affected cousin being affected from age 10. This onset age was later than in PLA2G6-related INAD[84] which begins before age 2 and typically causes death around age 7-10. Presenting signs in our patients were cognitive decline in one and walking difficulty due to leg dystonia in the other two. Onset was subacute resulting in severe motor and cognitive handicap within few years, when a full blown picture of extrapyramidal, pyramidal and cognitive/psychiatric features had developed. Cognitive features were characterized by frontal executive dysfunction, accompanied by personality changes and depression. One patient had bladder dysfunction and swallowing difficulties later in the course. Parkinsonism was reflected by marked reduction of uptake on dopamine transporter SPECT scan, which differed from the pattern seen in idiopathic Parkinson's disease, and improved with dopaminergic treatment. However, those on levodopa developed prominent and early dyskinesias.

Mutations in *PLA2G6* also cause NBIA and iron was present in all *PLA2G6*-related NBIA cases recently described.[85] In addition to marked cerebellar atrophy and progressive white matter changes, iron accumulation of the pallida (affecting medial and lateral portions) were also detected in further six *PLA2G6*-related

INAD patients.[84] In one, iron was not (yet) present on T2-weighted imaging (T2* scans not presented) 2 years after disease onset but prominent on both T2 and T2*-weighted MRI on 6-year follow up. Our finding of absent iron in the basal ganglia as confirmed by T2* weighted imaging, up to 12 years after disease duration, illustrates that a diagnosis of *PLA2G6*-related neurodegeneration should not only be considered in patients with dystonia-parksinsonism with brain iron accumulation but also those without.

VEPs and EMGs which are typically abnormal in classic young-onset INAD[83] were normal in our patient.

Pathologically, INAD is characterized by axonal degeneration with distended axons (spheroid bodies). However, there is evidence for pathological heterogeneity of INAD as cases with clinical and pathological features of INAD negative for *PLA2G6* mutations and in contrast *PLA2G6*-positve patients without spheroid bodies have both been described.[85] Pathological data regarding brain or peripheral nerves for patients with adult-onset *PLA2G6*-related NBIA are not yet available. However, a skin biopsy was normal in our case without evidence of spheroid bodies.

Clinically, our patients showed striking resemblance to Kufor-Rakeb syndrome[86] where disease onset was at age 12 -15 years, within the age range of our cases. However, none of the additional clinical features of facial-faucial-finger mini-myoclonus, visual hallucinations, or oculogyric dystonic spasms found in further Kufor Rakeb cases[87] were present in our cases. There also was clinical overlap with PKAN. However, lack of the "eye of the tiger" sign on MRI, the absence of oromandibular dystonia which is often severe in PKAN patients[75], as well as the absence of pigmentary retinopathy appears to distinguish the syndromes.[88]

Both mutations identified by us (p.R741Q and p.R747W) are novel: however, mutations at codon 741 have been reported recently (p.R741W in a child with INAD[89]) and the clustering of mutations at this part of the protein suggests this domain is critical for its function. Little, however, is known about this function. The clinical and pathological similarity of the syndrome caused by PLA2G2 deficiency to those caused by PKAN and *ATP13A2* deficiencies suggest that all three gene products may lie on a single biochemical pathway. Other genes involved in remaining similar syndromes may map to this same pathway.

Chapter 4 Transcranial magnetic Stimulation (TMS)

Transcranial magnetic stimulation (TMS) is a non-invasive method of stimulating cortical neurons. A magnetic field generator drives a current of approximately 200μs with a peak amplitude of 8,000 A through an induction coil placed on the scalp. The current creates a time-varying magnetic field perpendicular to the coil. The magnetic field penetrates the skull and then induces an eddy current parallel to the coil in the brain. This current is capable of stimulating the brain and can produce descending volleys in the corticospinal pathway which can be recorded using surface EMG from the appropriate muscles.

The motor "hot spot" is the area on the scalp over which TMS of a particular intensity produces the largest motor evoked response (MEP) from the target muscle. Due to ease of stimulation, the most commonly used target muscle is the first dorsal interosseus (FDI). Surface EMG is recorded from FDI during stimulation, and once the "hot spot" has been identified it is marked on the scalp.

A figure of eight coil is often used to provide a more focal stimulus than that obtained from a simple circular coil. If a figure of eight coil is held such that the TMS pulse causes current to flow in an posterior-anterior direction perpendicular to the central sulcus, then this tends to provide the lowest threshold for stimulation and appears to activate corticospinal neurons trans-synaptically.[90] As stimulation intensity is increased, a rising proportion of activation occurs directly.

The tendency for trans-synaptic activation means that the response to TMS is altered by the excitability of these synapses at the time of stimulation. Therefore

TMS is useful as a technique to explore the integrity and excitability of motor pathways, and can be applied before and after an intervention to determine whether a change in synaptic excitability has occurred.

I. Single pulse TMS measures

1. Resting and active motor thresholds.

Resting motor threshold (RMT) is defined as the minimum intensity needed to evoke an MEP of >50μV in 5 out of 10 consecutive trials in a relaxed muscle. Active motor threshold (AMT) can be defined as the minimum intensity (in % of maximum stimulator output) needed to evoke a MEP of >200μV in 5 out of 10 trials in the target muscle while it is voluntarily contracted. Typically feedback is given to the subject to maintain this voluntary contraction at a set level (about 20-30% of maximal contraction). Thresholds are a marker of excitability. They both mainly reflect axonal excitability; however synaptic excitability may also play a role in RMT production.

Spinal excitability also contributes to MEP size. To assess whether changes in MEP size are due to cortical or spinal changes, assessment of H-reflexes can be useful.[91] Assessment of other cortical measures like short afferent inhibition (see below) may also be helpful for this distinction.

2. Input/Output curves

Differences have been observed in the input/output relationship in response to TMS. In these experiments, the RMT for a particular individual is established, and then TMS pulses at increasing intensity of stimulation based on percentages

of RMT are delivered, and the size of the resulting MEP recorded. This provides an input/output curve where MEP size is plotted against magnitude of TMS intensity.

A difficulty with interpretation is that muscle activity directly influences the size of MEP produced from TMS of a given intensity of stimulation. Muscle activation in the target muscle or other adjacent or distant muscles (or even thinking about muscle activation) increases MEP size. This means that scrupulous monitoring of baseline EMG in the target muscle is required in order to prevent this possible artefact in experiments in hyperkinetic subjects, who may have a significant amount of involuntary muscle activity (such as dystonia or rigidity or tremor in parkinsonian patients or chorea). This unwanted preactivation may then result in false results of measures "at rest" and it may be advisable to study TMS measures during voluntary contraction instead which can be objectively measured. It may also be useful in future studies to give details about clinical findings in the studied limbs (for example whether rigidity was present rather than simply giving a total clinical score where it is not known which clinical finding predominated).

3. Silent Period

The silent period is a period of EMG silence that occurs following a TMS shock delivered over the representative area of cortex of a voluntarily contracting muscle. To achieved a constant sub-maximal muscle contraction auditory or visual feedback is usually given. A TMS pulse is then delivered over the motor hotspot at a certain intensity of e.g. 110-130% of RMT. A higher stimulus intensity possibly provides a more consistent result.[92] The temporary break in EMG activity is called the silent period. This can be measured in a variety of

ways, for example by measuring the interval between the onset of the stimulus artefact and the first recovery of EMG activity. Orth and Rothwell proposed that the ratio of the silent period and MEP amplitudes may be superior as it is independent from parameters like coil orientation and stimulation intensity.[92]

Studies in normal subjects typically find the silent period to be 100-120ms in length. Via examining the effect of GABAa and GABAb antagonists and agonists, It was proposed that the SP is a GABAb mediated process.[93,94] There is likely to be a small additional spinal component.[95]

4. Central Motor Conduction Time

The conduction time from the cortex to the spinal alpha motorneuron is referred to as central motor conduction time. To establish CMCT, the peripheral motor conduction time (spine to muscle) is being deducted from the cortico-muscular latency. For the latter measure, different methods have been described including transcutanous magnetic timulation of the spinal nerve roots.[96] In healthy subjects increasing stimulation intensity leads to a decrease in CMCT. Conduction may be slowed by demyelination, degenerative or ischemic changes of the fastest-conducting fibres, or decreased dispersion of the multiple corticospinal discharge which results in less temporal summation at the spinal alphaneuron.

II. Paired pulse TMS measures

1. Short Intracortical inhibition and facilitation

Kujirai et al [97] developed a paired pulse TMS technique which is thought to stimulate different populations of inhibitory and excitatory interneurons, and

provides measures of their excitability: intracortical inhibition and facilitation. The standard method explores the influence of a sub-threshold "conditioning" pulse on the size of the MEP produced by a subsequent "test" pulse. The intensity of the test pulse is usually set to achieve an MEP of about 1mV when given alone. The conditioning pulse is then given at different time intervals (interstimulus intervals, ISIs) prior to the test pulse.

In studies with normal subjects, the conditioning pulse given 1-5ms prior to the test pulse causes a reduction in the resulting MEP.[97] This effect is known as short interval intracortical inhibition (SICI). The effect is enhanced by GABAa agonists, NMDA receptor blockers and dopamine agonists and is blocked by dopamine antagonists.[98,99] It is proposed that SICI is a GABAa mediated pathway that has an inhibitory influence of corticospinal tract excitability.

There is a cross-over or intermediate period of response when the conditioning pulse is given between 6 and 9ms prior to the test pulse, where little effect is seen on the resulting MEP. At interstimulus intervals (ISIs) of 10-20ms an increase in the size of MEP is typically seen, a phenomenon known as short intracortical facilitation (ICF).[97] It can be modified by rTMS independently of SICI indicating that different pathways underlie the two phenomena.[100] The mechanism of ICF is unclear at the present time. Currently it is thought most likely to be a glutamate mediated event. [98]

a) SICI recruitment curve

SICI can be influenced by the intensity of the conditioning pulse. Thus, rather than testing effects of different ISIs, effects of varying stimulation intensities of the conditioning pulse at a fixed ISI are assessed. SICI is recordable using a

conditioning pulse intensity of 60% RMT at an ISI of 3ms. The magnitude of the effect increases as the intensity of the conditioning pulse is increased, and reaches a maximum at approximately 90% of RMT or 80% AMT. [97,101] Further increases in intensity lead to progressively less SICI. Although less certain, it may be that the optimum intensity for producing ICF is slightly higher than that for SICI.

2. Short afferent inhibition

A sensory (afferent) conditioning stimulus, such as median nerve stimulation or digital nerve stimulation, delivered to the contralateral hand can inhibit the motor cortex as measureable by decreased motor evoked potential amplitude. Inhibiton that occurs at ISIs of 20 ms between the conditioning sensory stimulation and the test TMS is referred to as short latency afferent inhibition (SAI).[102,103] Inhibition occuring at longer ISIs of ~200 ms is referred to as long latency afferent inhibition (LAI). [104]

III. Plasticity of the nervous system

The nervous system is able to adapt to (internal or external) stimuli and to remain in that changed state until further stimuli occur. In other words: it is plastic. The ability of the nervous system to plasticity can be demonstrated experimentally, for example using TMS methods. Plasticity of the motor system presents as change in motor "maps" in the primary motor cortex (M1) in response to pathological or physiological interventions.[105] Practicing and learning of motor skills induces plastic changes and expansion of cortical representation of the body parts involved in the motor task in primates and humans.[105] Appropriate

TMS-challenge can also result in such a change of the functional organisation which outlast the period of conditioning (the TMS pulse). This is the case even if the pulses are delivered at sub-threshold intensities (i.e intensities of stimulation that produce no recordable muscle activation). It is thought that these changes are a form of long term potentiation (LTP) and long term depression (LTD) (see under mechanisms).

1. Repetitive transcranial magnetic stimulation

Various protocols of rTMS have been devised for the induction of long-term changes in cortical excitability. However, it was found that high frequency stimulation (20Hz and above) comes with the risk of inducing seizures in humans.[106] Subsequently, international safety guidelines were introduced which restrict the frequency of stimulation that may be applied to human subjects.[107]

Low-frequency rTMS, most commonly used at a frequency of 1 Hz, delivered at 90% resting motor threshold applied for 20-30 minutes produces an LTD-like effect which can be measured for about 30-40 minutes from the end of conditioning.[108-110] Electrophysiologically, this LTP effect can be measured as decrement in the amplitude of motor evoked potentials as compared to MEP size prior to rTMS application.

In contrast, high-frequency rTMS, e.g. 5Hz rTMS, induces LTP-like effects in human cortex. To avoid coil overheating or other technical problems, such stimulation is given in blocks rather than one continous long train.[111,112]

There have also been studies exploring the effect of yet higher frequencies at higher intensities, including 20 pulse trains at 20 Hz and 150% RMT.[113]

Similarly to 5Hz rTMS, these protocols produces short lasting (seconds to minutes) increments in cortical excitability.

2. Theta-burst TMS

Recently, Huang et al[114] developed a protocol of rTMS, called theta burst stimulation (TBS), which produces longer lasting effects of 60 min following a brief stimulation period of 90-120 sec. Patterns of TBS include the intermittent theta burst stimulation pattern (iTBS) and continuous theta burst stimulation paradigm (cTBS). Pulses of stimulation are given at 50 Hz, repeated every 200 ms. In the intermittent theta burst stimulation pattern (iTBS), a 2 s train of TBS is repeated every 10 s for a total of 190 s (600 pulses). In the continuous theta burst stimulation paradigm (cTBS), a 40 s train of uninterrupted TBS is given (600 pulses).

The direction of change in synaptic efficiency (facilitatation following iTBS vs inhibition following cTBS) depends on the pattern of TBS delivery. Facillitation is seen following iTBS (similar to high frequency rTMS), while continuous TBS has inhibitory effects (similar to low-frequency rTMS).

Figure 4.1 Graphical illustration of TBS paradigms (A) and their effects on MEPs (B). (from Huang et al[114])

3. Interventional Paired associative Stimulation (IPAS)

Plastic changes in the excitability of motor cortical pathways in humans that outlast the period of stimulation by minutes to hours can also be produced using paired pulse stimulation protocols.[115] Here, a sensory afferent (via direct stimulation of a nerve) and the homologous cortical efferent (via a single TMS pulse over the corresponding area of motor cortex) pulse are given together.

Dependent on the timing of the pulses, increases or decreases in cortical excitability can be produced (similarly to SICI and ICF). For example, if median nerve stimulation is given 25 ms before TMS, low-frequency median nerve stimulation, paired with suprathreshold transcranial magnetic stimulation (TMS) over the optimal site for activation of the abductor pollicis brevis (APB) muscle typically induces a long-lasting increase in the excitability of corticospinal output neurons.

On the other hand a gap of 10ms between sensory stimulation and cortical stimulation causes a decrease in cortical excitability.[116]

In humans, the effects of IPAS can be blocked by NMDA antagonists such as dextromethorphan.

4. Behavioural effects of TMS- or DCS-induced plasticity

In addition to changes in electrophysiological measures, e.g. MEP size, plasticity-inducing TMS or DCS protocols can result in alterations in behaviour. For example, serial reaction time tasks may be affected by rTMS. In recent years effects of rTMS on behaviour has been studied not only in healthy volunteers but also in patients with varies neurological and psychiatric diseases with the aim of

improving symptoms. [117] (see below for effects of rTMS application in selected movement disorders)

5. Mechanisms of plasticity

The mechanisms of plasticity are not fully understood. Brain slice experiments allow measuring field potentials (FP) and the response to stimuli. Changes in the FP in response to conditioning pulses provide a direct measure of changes in synaptic strength. This approach allows manipulation of conditioning stimuli and physiological conditions in order to better understand the mechanisms of synaptic plasticity. Both pre- and postsynaptic mechanisms have been described. Changes at the pre-synaptic level are related to changes in the amount and/or probability of transmitter release (for example calcium influx),[118] availability of vesicles, growth of the bouton, or number of boutons and result in short lasting changes in synaptic efficiency (lasting milliseconds to seconds). At the post-synaptic level, plasticity occurs via glutamate receptors. While high frequency direct electrical stimulation causes long-term potentiation (LTP) as shown in animal brain slice preparations[119], low-frequency stimulation produces long-term depression.[120]

LTP, in general, is an NMDA receptor-dependent process, so that pharmacological[121,122] or genetic[123] blockade of these receptors leads to reduction of after-effects of plasticity protocols with failure of LTP induction.

Chapter 5 TMS in Genetic Movement Disorders

TMS has been used to explore various neurological and psychiatric disorders, however, in many of the studies the cohorts were etiologically and/or clinically hetereogenous (for example, Parkinson's disease is probably a heterogenous disorder with different subtypes). Consequently, results have varied and have sometimes been inconclusive.

With identifaction of genes underlying diseases there has been increasing interest in using TMS for assessment of genetic conditions as patients can be recruited based on their molecular diagnosis to ensure a homozygous study cohort. A relatively small number of studies based on such concept have been published including some focusing on non-movement disorder patients[124-128] In the following the current knowledge of TMS in genetic movement disorders will be reviewed. A summary is given in the Appendix (table 5.1)

I. TMS in Genetic Parkinson's disease

There has been extensive research into Parkinson's disease using TMS; however, except for two studies, patients had the idiopathic form. Only very recently, two studies assessed parkin-related parkinsonism, one study focussed on patients, the other one reported data obtained from carriers. To date no other monogenetic forms of Parkinson's disease (PINK1, DJ1, LRRK2) have been explored using TMS.

In idiopathic PD (see Cantello[129] et al for review), the main findings include shortening of CSP, reduction in SICI, but normal thresholds, normal ICF and normal CMCTs. Levodopa was found to restore the abnormal measures.

In the study on parkin disease with four patients, De Rosa et al[130] found evidence of prolonged CMCT. In detail, CMCT was slow in two patients in both upper and lower limbs. A further patient had abnormal CMCT in the arm and the fourth patient abnormal CMCT in the leg. Further findings were increased MEP thresholds in two patients and decreased thresholds in one patient. Two patients had shortened CSP. However, other aspects of cortical excitability, in particular integrity of intracortical inhibitory systems, have not been studied, and it is unclear whether parkin patients differ from idiopathic PD patients.

Electrophysiological studies of carriers are also limited to one study. Recently, Baumer and colleagues[131] reported abnormal short afferent inhibition, a measure of sensorimotor integration and cholinergic activity, whereas short interval intracortical inhibition, mediated mainly by $GABA_A$ interneurons, was normal,[132] Whether this reflected a form of "compensation" or a direct consequence of the underlying dopaminergic deficit was unclear.

II. TMS in Genetic Chorea

Numerous studies have investigated familial choreas using TMS; however to date reports are only available for HD, and not for the other forms of genetic chorea some of which have been outlined above. Notably, most of these studies date from the time prior to identification of the HD gene and diagnosis was made based on clinical features. Thus, the studied cohorts may have been gnetically heterogenous. Only recent studies enrolled patients with molecularly confirmed diagnoses. This issue will be considered in the following. Furthermore, except for one study[133], data did not reflect electrophysiological changes over time.

1. Single Pulse TMS in HD

a) Thresholds and Input/Output Curves in genetically proven HD

Resting motor thresholds were normal in two studies with 17 and 11 patients with genetically proven HD.[134,135] AMT was also assessed in one of these studies and found normal. [134]

Similar results had been suggested by previous historical reports based on a clinical diagnosis[136,137], however, Meyer et al[138] had found abnormally high thresholds and reduced MEP amplitudes in up to 72% of 34 patients with manifest HD and abnormalitites correlated with duration of motor symptoms. However, it needs to be kept in mind that these studies were undertaken prior to identifaction of the gene.

b) Silent period in in genetically proven HD

While selected trials revealed normal silent period duration in 17 patients with genetically proven HD, other trials contained significantly prolonged silent period durations with a larger variance and range compared to controls.[135,139,140] Lefaucheur et al reported deterioration of silent period measures over time after studying 20 patients with gene-proven HD. Mean age of his cohort was 42 years, mean CAG repeat 45,3 (range 41-51) and mean duration of evolution 6±2 years. He calculated an annual slope of -27±10ms for silent period duration.

The prolonged SP in HD opposes findings in PD where SP is generally shortened.

c) CMCT in HD

Central motor conduction time was found normal in 32 patients with clinically-diagnosed Huntington's disease and 14 subjects at risk. [137] CMCT was also normal in patients with early gene-proven HD.[141]

2. Paired pulse TMS masures in HD

a) SICI/ICF in in genetically proven HD

Abbruzzese et al[142] reported reduced cortico-cortical inhibition at intervals of 2-5 ms and reduced ICF at intervals of 10-25 ms in nine patients with genetically proven HD with a mean disease duration of 6.2 (±4.1) years. Analysis revealed an inverse correlatation of inhibition with onset age and a positive significant correlation with dyskinesia ratings but not with UHDRS scores.

Two genetically proven pre-symptomatic carriers of who, however, no further details like CAG repeat length or predicted years to onset are known showed a "time course of conditioned MEP changes at paired cortical stimulation similar to that of normal controls".[142]

In a recent study, Nardone et al assessed twelve patients (mean age 33.5 years) with a molecular diagnosis of HD. Patients were in early disease stages, classified as stage I according to Shoulson and Fahn[143]. UHDRS scores ranged from 0 (n=4) to 13. The authors found reduction in ICF, while other measures were normal. [141]

b) Short afferent inhibition

Short afferent inhibition has not been studied in HD.

3. Plasticity TMS measures in genetic chorea

a) rTMS

Lorenzano et al [134] applied 5Hz rTMS at a suprathreshold intensity of 120% resting threshold over the M1 area in eleven patients with a molecular diagnosis of HD. Mean age of patients was 56±10.2 years (range 40-69), mean disease duration was 6±2 years (range 3-10). HD manifested with typical chorea. UHDRS scores ranged from 11-55 (mean 26±15). However, rTMS resulted in progressive increment of MEP size in controls, however, no changes were seen in HD patients. In both groups, cortical silent periods invariably lengthened. [134]

Recently, Crupi et al[144] probed LTP-like plasticity motor cortex in eight genetically proven (mean UHDRS 31). Two paradigms were performed. Motor cortex plasticity was assessed using paired associative stimulation (PAS) at an ISI of 25 ms. Secondly, brainstem plasticity was studied using pairing of an high-frequency train of electrical stimuli over the right supraorbital nerve (SO) coinciding with the R2 response elicited by a preceding SO stimulus.[145] The authors found impairment of both cortical and brainstem LTP-like plasticity in their symptomatic HD patients.

4. Other electrophysiological measures in HD

a) Blink reflex in HD

R1 components reflect oligosynaptic pons pathways. R1 responses are normal in patients with a clinical diagnosis of HD. [146] [147]

The R2 component of the blink reflex which is mediated by polysynaptic brain stem pathways has been found abnormal in 11 patients with clinically diagnosed Huntington's chorea who showed prolonged latencies ipsilaterally and contralaterally.[146]

Studies also revealed changes in amplitude and duration of R2; however with somewhat inconclusive results with either increased or decreased measures compared to healthy subjects.[146] [148] This may reflect high variability of R2 responses in HD.

Finally, patients also showed greater habituation of R2 responses. [148-150] These abnormalities correlated with severity of facial chorea. [146] This indicates depression of the blink reflex in HD due to reduced excitability of polysynaptic networks within the brain stem.

b) Masseter reflex in HD

Cruccu et al[151] found normal masseter inhibitory reflex latencies, depth of suppression, duration and recovery cycle to paired stimuli, in patients with clinically diagnosed Huntington's chorea.

c) H reflex in HD

Priori et al[152] reported increased facilitation of the test H reflex recovery cycle in the flexor carpi radialis at conditioning test intervals of 10-200ms in 16

genetically proven HD patients (with a mean clinical severity score of 10.4±1.7 on the Marsden& Quinn Chorea Evaluation scale (max score 24)). Abnormalities were most prominent at 40-60ms. The authors compared those to cortical inhibitory measures to determine the origin of the abnormal inhibition patters, however, cortical inhibition was found normal (see above).

d) Reciprocal inhibition in HD

Priori et al[152] also studied reciprocal inhibition in his patients with clinically-diagnosed HD patients and found a significantly decreased presynaptic phase. This reached a minimum at the conditioning test interval of 20ms.

e) Bereitschaftspotentials in HD

Absence of pre-movement Bereitschaftspotentials preceeding choreic movements led Shibasaki et al[153] to the conclusion that choreic movements of HD patients were indeed involuntary. Again, this study was done based on a clinical diagnosis of HD.

5. Summary of TMS in genetic Chorea

In summary, TMS studies suggest reduced cortical excitability in HD based on the findings of reduced amplitudes in cortical components of SEPs[154], long-latency reflexes,[155,156] premotor potentials,[157] – in the absence of changes in subcortical levels. [134] Deterioration over time affected blink reflex latency, long-latency reflexes, SEP parameters (N20 and N30 presence).[133] There may be variability of silent period duration and abnormalities in SICI suggesting alterations of GABA-ergic mechanisms. Progression of abnormalities of silent

period duration over time was suggested.[133] Plasticity was reduced in HD patients.

III. TMS in Genetic Dystonia

1. Clinical Overview

Dystonia is defined as "a syndrome of sustained muscle contraction, frequently causing twisting and repetitive movements or abnormal postures" as produced by the Scientific Advisory Board of the Dystonia Medical Research Foundation.[158] Dystonic syndromes can be classified according to aetiological cause with the main separation being "primary" versus "secondary/heredodegenerative".[158] The aetiological classification furthermore includes paroxysmal dystonias and the "dystonia plus" syndromes, dopa-responsive dystonia (DRD) and myoclonic dystonia. These "dystonia plus syndromes" are conditions where dystonia occurs together with other movement disorders, but where there is no secondary or neurodegenerative cause.[159]

Familial forms of dystonia have been recognised for many years, and genetic investigation of such families have revealed a number of possible loci and in some cases particular gene mutations. These are largely summarised in the "DYT" gene classification system, which currently extends from DYT1 to 16.[160]

a) DYT1 Dystonia

As for the "pure" dystonias, DYT, due to a single GAG deletion in torsin A (TOR1A gene) on chromosome 9q34,[161] classically presents with young-onset lower limb dystonia which later spreads to become generalized. The cranio-

cervical is usually spared. Inheritance is autosomal dominant. An increased prevalence in the Ashkenazi Jewish population has been noted thought to be due to a "founder effect".

DYT1 dystonia is particularly interesting in the context of the proposed projects as penetrance is reduced and clinical symptoms in DYT1 mutation carriers are present only in approximately 30%, and almost all those who are going to manifest symptoms will do so before the age of 25. DYT1 mutation carriers therefore present a unique opportunity to the researcher with an interest in the pathophysiology of dystonia and consequently these subjects have been particular given attention. Functional imaging studies in DYT1 have provided clues that clinically normal individuals who carry the DYT1 mutation have abnormalities in brain structure and function. TMS data in unaffected DYT1 carriers are summarized below.

b) Dopa-responsive Dystonia (DRD)

A rare form of dystonia is DRD (Segawa syndrome). These patients typically have young-onset limb dystonia and in many cases additional parkinsonism and mild pyramidal signs. Diurnal fluctuations of symptoms is reported in a proportion of patients with worsening of symptoms throughout the day.[162] Phenotypic variability is common, but in almost all cases a dramatic and sustained response to levodopa is seen. For the classic form mutations in the gene encoding guanidine triphosphate cyclohydrolase 1 (GTPCH1) could be identified, GTPCH1 is a rate limiting step in the metabolism of tetrahydrobiopterin, itself an essential co-factor in the production of dopamine from tyrosine [163, 164] Mutations in TH4 cause a more severe phenotype.

Two studies have investigated DRD using TMS.

c) Myoclonic Dystonia (DYT11)

In myoclonic dystonia, familial early childhood onset dystonia (typically affecting the neck and arms) is accompanied by myoclonus in a similar distribution.[165] The myoclonic jerks are described as "lightning jerks", and alcohol responsiveness is common.[165] Recently, mutations in the epsilon sarcoglycan gene (SGCE) have been found in a proportion of patients with myoclonic dystonia.[166] The gene shows maternal imprinting, meaning that offspring receiving a mutant gene from their mother will almost never show symptoms, in contrast to those who receive a mutant gene from their father, where penetrance is almost complete.[167] To date one TMS studies focussed on myoclonic dystonia patients.

d) Paroxysmal Dystonia/ Dyskinesias

Furthermore, genetic causes have been identified for some of the paroxysmal dyskinesias /dystonias – a condition where dystonia occurs in attacks whereas patients do not show any signs interictally. Based on the classification by Demirkiran and Jankovic[168] four formes can be distinguished: paroxysmal kinesigenic dyskinesias (PKD), paroxysmal non-kinesigenic dsykinesias (PNKD), paroxysmal exercise-induced dsykinesias (PED) and paroxysmal hypnogenic dsykinesias (PHD) according to triggering factors (sudden movement (PKC), prolonged exercise (PED), emotions/ fatigue (PNKD) and sleep (PHD)). Responsible genes have recently been identified for PNKD and PED. In the former mutations have been found in the MR1 gene on chromosome 2.[169] In the latter, familial and sporadic cases were recently found to carry

mutations in GLUT1, encoding for a glucose transporter which provides the CNS with glucose (see above under Clinical Experiments). In PHD mutations on chromosomes 15q24 and 20q13.2-13.3 coding for the <a>4 and <b>2 subunits of nicotinic acetylcholine receptors (nAChRs) have been identified.[170]

Two TMS studies focussed on paroxysmal dyskinesia, however diagnosis was made on a clinical basis and not genetically confirmed.

2. TMS in Dystonia

A wealth of electrophysiological and imaging data exists in patients with dystonic syndromes whereas data for genetic dystonia are limited to studies in DYT1 dystonia, DRD and paroxysmal dyskinesia /dystonia. Three studies report data of DYT1 dystonia, including one assessing effects of deep brain stimulation. TMS data of dopa-responsive dystonia and paroxysmal dyskinesias are limited to two studies each and there is one study on myoclonic dystonia. No data are available for any of the other DYT-realated forms of dystonia.

a) Single pulse TMS measures in Dystonia

(i) Thresholds in Dystonia

Abnormalities in non-genetic dystonia patients include a significantly enhanced input/output curve, such that MEP size is significantly larger for a given input compared to control subjects [171172] interpreted as increased excitability of the motor system, although no differences have been found in thresholds for activation of muscles in sporadic dystonia subjects compared to controls. Similarly, thresholds were normal in genetic dystonias including DYT1

manifesters and carriers[173], DRD[174] and paroxysmal dystonia. [175] Increased active motor thresholds have been reported in five gene-proven DYT11 gene carriers.[176]

(ii) Silent Period in Dystonia

In dystonic subjects, including DYT1 manifesting patients and non-manifesting subjects, a number of studies have found a shortened silent period[177-179] suggesting deficits of GABAb circuits,[98] whereas the silent period was found normal in DRD[180] and paroxysmal dyskinesias.[175,181]

b) Paired pulse TMS measures in Dystonia

(i) SICI and ICF in Dystonia

SICI appears to be reduced in patients with primary dystonia including DYT1 cases,[177,173,178,182] DRD[180] and paroxysmal dyskinesias.[181] SICI was also reduced in DYT1 carriers.[179] This has been interpreted as a failure of probably GABAa-dependent[98] inhibitory control of motor pathways which could lead to problems in focusing desired movement and could lead to unwanted muscle activity.[177] As with input-output experiments, measurement of SICI (and ICF) is hampered by muscle contraction – it will tend to reduce SICI and ICF.[183] However, reductions in SICI have been demonstrated using target muscles that are not involved by dystonia (e.g. FDI in patients with cervical dystonia).

Others found normal SICI in other genetic dystonias including gene-proven myoclonus dystonia, DRD and paroxysmal dyskinesias. [174-176]

ICF is normal in genetic dystonias.[179-181]

(ii) Short Afferent Inhibtion in Dystonia

Short afferent inhibition (SAI) is typically normal in patients with (sporadic) dystonia. Long afferent inhibition (LAI) was found abnormal in patients with writer's cramp, but not in cervical dystonia, suggesting a different mechanism from LAI. [184,185]

SAI was normal in five gene-proven patients with myoclonus dystonia. SAI has not been assessed in other genetic dystonias.

c) Plasticity in Dystonia

rTMS, theta burst stimulation and IPAS have been used to study plasticity in dystonic syndromes. With respect to genetic dystonias, only DYT1 has been assessed [173,178,186]. Here, rTMS, rTMS paired with DCS[187] and PAS have been explored.

In both non-genetic and genetic dystonia plasticity is increased. Interventional paired associative stimulation (IPAS) produced more stimulation-induced facilitation of MEP amplitudes in focal arm dystonia patients[188]; 1Hz rTMS prodcued more widespread changes in the cortex of patients as demonstrated by PET.[189] In DYT1 patients 1 Hz rTMS over the premotor area produced a significant increase in reciprocal inhibition (affecting the third and possibly the first phase), while no changes were observed in controls.[178] Theta burst stimulation produced a significantly prolonged response in eight DYT1 patients compared to healthy subjects (see Figure 5.1).[173] Notably, in DYT1 carriers [173]theta burst stimulation resulted in a reverse (or: lack of) response.

Figure 5.1 The normalized motor evoked potential (MEP) size at baseline and following repetitive transcranial magnetic stimulation (rTMS) in healthy subjects, manifesting DYT1 mutation carriers (MDYT1), subjects with torticollis, and nonmanifesting DYT1 mutation carriers (NMDYT1). Error bars indicate the standard error of the mean. (from: Edwards et al[173])

d) Other Electrophysiological Measures in Dystonia

(i) Blink Reflex in Dystonia

The blink reflex has been found enhanced in certain types of non-genetic dystonia (blepharospasm, cervical dystonia, generalised dystonia) such that the R2 component is large even at ISIs of 250-500ms.[190-193] Similarly, patients with dopa-responsive dystonia were found to have an abnormal blink reflex recovery cycle at 200, 500 and 1000ms[180] when studied "off" treatment. The authors also found that the significant increase in the excitability of the blink reflex recovery cycle decreased with levodopa treatment. Similar normalization has been

reported for idiopathic PD.[194] Blink reflex data for other genetic forms of dystonia including DYT1 and paroxysmal forms are not available.

(ii) Reciprocal Inhibition in Dystonia

Although primary dystonia by definition does not present clinically with signs of corticospinal or radicular dysfunction, electrophysiological testing has revealed deficits in spinal reflex control. Compatible with the original description of Nakashima et al.[195] in non-genetically characterised dystonia, spinal reciprocal inhibition was normal in DYT1 patients in respect to the first phase of inhibition, however, later phases were reduced[179]. Nonmanifesting DYT1 carriers had normal spinal reciprocal inhibition.[179]

e) Summary of TMS Findings in Dystonia

In primary dystonia, both non-genetic and genetic forms, the overall impression is of an over-excitablity and a reduction in motor inhibitory circuit activity/function evident at many levels of the nervous system, but most likely with its origins in the basal ganglia. Secondly, there is evidence of abnormal plasticity in dystonia with increased susceptibility to undergo changes in synaptic effectiveness, present in manifesting patients. Abnormal plasticity of the sensorimotor system in dystonia is compatible with previous theories of dystonia, such as lack of "surround inhibition" {Mink, 2003 1878 /id;Sohn, 2004 770 /id;Sohn, 2004 771 /id} or disordered sensory "gating" of movement.[196] The subclinical physiological deficit in non-manifesting carriers are not as widespread as those seen in manifesting patients. Notably, non-manifesting gene carriers appear to be resistant to a plastic force like rTMS, which has been interpreted as protective, compensatory mechanisms.This would be consistent

with the hypothesis that additional genetic/environmental insults are necessary to produce clinical dystonia in gene carriers.

Sensory system function is certainly not normal in dystonia, but it is still unclear whether this is a primary feature of dystonia or its consequence. Abnormalities are also present in non-manifesting DYT1 subjects and may represent another form of endophenotype.

IV. TMS in Genetic Myoclonus

1. Definition and Classification Systems of Myoclonic Disorders

Myoclonus is characterized by a sudden, brief shock-like jerk due to involuntary contractions (positive myoclonus) or inhibition of muscular tonic contraction (negative myoclonus). Myoclonus can be classified by etiology which may include degenerative, genetic, metabolic or other causes.

As for genetic forms of myoclonus, this includes the big group of progressive myoclonic epilepsies (PME) which comprises Unverricht Lundborg disease[197] (due to mutations in the cystatin B gene EPM1 on chromosome 21q22.3), Lafora's disease (due to mutation of the EPM2A gene on chromosome 6q24; or EPM2B gene on chromosome 6p22), myoclonic epilepsy associated with red ragged fibres (MERRF; due to mutations in the MTTK mitochondrial gene); Sialidosis (due to neuraminidase deficiency) and DRPLA (due to triplet repeat expansion in the DRPLA gene). In children presenting with the combination of myoclonus and epilepsy the diagnosis of juvenile myoclonic epilepsy (JME) should be considered. Although JME is mostly sporadic, a few genes have been identified for this genetically hetereogenous disorder in individual cases. This

includes mutations in the GABRA1 gene,[198] mutations in the CACNB4,[199] mutations in the chloride channel-2 gene (CLCN2)[200] and mutation in the GABRD gene.[201] Other genetic causes of myoclonus include myoclonus dystonia due to mutations in the epsilon sarcoglcan gene which is discussed above under dystonic syndromes and those causing a more complex phenotype with prominent dementia (e.g. Rett syndrome or Angelman syndrome).

Another classification system of myoclonus disorders is by anatomical origin into cortical, subcortical, brainstem and spinal cord myoclonus or myoclonus related to the peripheral nervous system. For localization of the origin of the mycolonic jerks, electrophysiological techniques can be helpful. This includes EMG studies, EEG studies with back-averaging of jerks and pre-movement EEG potentials, and SSEP recording.

TMS data are overall limited for myoclonic conditions and more so for those with genetic causes. One reason for this may be that, as mentioned above, many of the genetic myoclonic syndromes manifest with epilepsy, and caution is to be used when these patients are studied and hence most centers may regard epilepsy as a contraindication for TMS.

2. TMS in genetic Myoclonus

Few data are available on patients with PME and patients with JME. However, none of the TMS studies assessed genetically proven cohorts of patients, but the diagnoses were made on clinical grounds. Aim of these studies was to distinguish between different clinically overlapping syndromes[202] or assess effects of antiepileptic treatment on electrophysiological measures.[203]

a) Single pulse TMS measures in Myoclonus

(i) Thresholds in Myoclonus

Resting motor thresholds were normal in patients with JME.[204,205] However, in PME findings are more controversial with normal[206] or increased thresholds.[207] Such increased thresholds may be a result of anticonvulsant treatment and can also be found in patients with sporadic generalized epilepsy.

(ii) Silent Period in Myoclonus

Silent period has been reported normal in 12 patients with PME [206] and patients with JME[205] as opposed to shortened CSP in myoclonus syndromes where dementia is prominent such as Angelman syndrome, Rett syndrome or Corticobasal Degeneration.[208]

b) Paired pulse TMS Measures in Myoclonus

(i) SICI, LICI and ICF in genetic Myoclonus

At ISIs of 2-5 ms, six PME patients showed either no or reduced inhibition.[209] This is in line with findings in JME[202,207] and non-genetic forms of myoclonus (such as multifocal cortical myoclonus, multifocal and bilateral myoclonus, generalized cortical myoclonus;[210] focal epilepsy and cortical myoclonus).[211]

However, a dissocitation between PME and JME can be found with respect to long intracortical inhibition (LICI) which is normal in JME but reduced in PME. As mentioned above, in both these conditions, CSP is normal. The dissociation of CSP and LICI in PME is noteworthy as both these measures are tought to reflect GABA B activity. (see Lefaucheur[208] for review)

The loss of inhibition was associated with the spread of myoclonus irrespective of whether epilepsy was present or not[210] which may argue for partly different pathophysiological mechanisms underlying mycolonic bursts and epileptic discharges.[208]

ICF is normal in both JME and PME. [204,205,207]

(ii) Short Afferent Inhibition in Myoclonus

In PME, a subcortical origin of the myclonic activity has been suggested. Clinically, myoclonic jerks can be triggered by stimuli including touch in PME patients and giant somatosensory evoked potentials can be detected in such patients. Similarly, abnormal SAI measures in PME reflect the involvement of sensorimotor system.[209,212,213]

In contrast, jerks cannot be elicited by audio or sensory stimulation in JME and here SAI is normal. [204]

c) Plasticity in Myoclonus

(i) rTMS

Using low-frequency rTMS Fregni et al[203] found favorable results with reduction of myoclonus-related activity in 15 patients with JME and there was correlation with plasma valproate levels in so far as patients with low levels showed a significant inhibitory effect to rTMS whereas in patients with high levels rTMS increased the corticospinal excitability significantly. Single cases of secondary myoclonus and epilepsy with favourable response to rTMS have also been reported [214,215]

A long-latency response to peripheral stimulation and an exaggerated facilitatory effect of peripheral stimulation on the motor evoked potential was present in subjects with PME,[212] suggesting an exaggerated effect of afferent input on motor cortical excitability in PME.

d) Summary of TMS in Myoclonus

Overall, TMS data on myoclonic conditions are rare (see Lefaucheur[208] for review); more so in genetic myoclonus. However, it appears that within the group of myoclonic epilepsies, PME may cause wider alterations compared to other forms.

It appears that myoclonus disorders with origin of the jerks on a cortical level are mainly characterized by lack of inhibition in the sense of reduced SICI, while silent period measures are normal. The dissociation of CSP and LICI in PME is noteworthy as both these measures are tought to reflect GABA B activity. Low-frequent rTMS may be beneficial to reduce involuntary activity.

V. TMS in Genetic Ataxia

1. Clinical overview

The term ataxia which originats from the Greek word for "without order" (or incoordination) refers to disturbances in the control of body posture, motor coordination, speech control, and eye movements. Genetic forms of ataxia are classified into dominant, recessive and x-linked forms. The group of dominant ataxias comprises among others the spinocerebellar ataxias (SCAs) and to date 28 genetic loci have been detected or reserved. Of these, SCA6 is considered a

"pure" cerebellar disorder, whereas the other genetic SCAs may present with other symptoms including movement disorders or neuropathy. A second group of autosomal dominant ataxias entails the episodic forms type I (chromosome 12p13) and II (chromosome 19p13).

The group of recessive forms comprises Friedreich Ataxia, Ataxia Teleangiectasia and its phenocopies, Ataxia with oculomotor aparaxia I and II (due to mutations in aparataxin on chromosome 9p13.3 and senataxin on chromosome 9p34), Ataxia with neuropathy I (SCAN1) and others.

2. TMS in genetic ataxias

With respect to TMS, it is the common forms of ataxias which have mainly been investigated. In addition to several historical reports based on clinical, histological or electrodiagnostic diagnoses (e.g Cruz-Matinez and Palau [216]), recent studies based on a molecular diagnosis have been published and these will be reviewed below. Genetically-proven cohorts investigated by TMS include SCA1, 2, 3 and 6 of the dominant ataxias and the recessive syndrome of Friedreich's Ataxia. Other genetically-proven ataxia syndromes have not yet been studied.

a) Single pulse TMS measures in genetic ataxia

(i) Resting motor thresholds

The mean resting motor threshold was normal in seven patients with SCA6[217] with normal MEP recruitment curves.[218] RMTs were also normal in SCA3.[219]

In SCA1 reting thresholds were increased in the upper limbs,[219,220] although the number of subjects was small (n=3) in one of these studies.

In SCA2, resting motor thresholds were normal.[219] However, around the same time Restivo et al [221] reported significantly increased resting motor thresholds in the lower limbs in patients with SCA2; whereas no differences were found in upper limbs, in line with the reports by Schwenkreis.[219]

In Friedreich's Ataxia, some authors have found significantly higher mean phosphene threshold and motor threshold values in patients than controls which correlated with size of the GAA1 expansion.[222] Other groups found normal resting motor thresholds.[219]

(ii) Silent period in genetic ataxia

While ipsilateral silent period was normal in SCA6,[218] contralateral silent period was found significantly prolonged in these patients compared to controls. [218]

CSP duration in both arm and leg muscles was significantly longer in SCA2 patients (as well as non-genetic ataxia) than in controls.[221,223] There was a significant positive correlation between disease duration and CSP prolongation in SCA2 but no correlation between age, age at onset and CSP duration emerged.

Silent period measures were normal in SCA3 and Friedreich's Ataxia.[219]

(iii) CMCT in genetic ataxia

No differences were found in mean CMCT as tested by the F-wave method between normal controls and SCA6 patients. [217,219] Similarly, normal CMCT measures were found in SCA2 and SCA3; whereas CMCT was prolonged in SCA1 and Friedreich's Ataxia.[219,220] Clinical measures such as presence of

pyramidal signs, neuropathy and severity and duration of cerebellar disease correlated with CMCT slowing.

b) Paired pulse TMS in genetic ataxia

(i) Short Intracortical inhibition and facilitation

In SCA6 and SCA1 both SICI and ICF were normal.[219]

In SCA2 and SCA3, SICI was normal.[221] However, ICF at 8, 10, 15 and 20ms/30ms was reduced in SCA2 and SCA3 at all intervals which correlated with disease severity in one of the studies [219,221]

In Friedreich's Ataxia, SICI and ICF were normal.[219]

(ii) Short afferent inhibition in genetic ataxia

In SCA6, both short-latency afferent inhibition and long-latency afferent inhibition were found normal. [218]

c) Plasticity of the nervous system in genetic ataxia

(i) rTMS

A small study (n=4) investigated the possible therapeutic effects of 5Hz rTMS over the cerebellum on clinical parameters in four patients with genetic ataxia. [224] Subjects were 2 patients with SCA6, one patient with SCA1, one patient with SCA7. Ten pulses were delivered over each hemisphere and the middle of the cerebellum on 21 consecutive days. Clincal parameters including the time

required for walking 10 meters, and the ability to perform tandem gait and to keep body balance improved significantly. Other cerebellar signs like nystagmus, dysarthria and incoordination remained unchanged after rTMS application. Blood flow increased in the cerebellar hemispheres, putamina and pontine base as demonstrated by SPECT.

Although this is a small pilot study with four patients only, the results are beneficial and large studies would be interesting to investigate the effect of rTMS on genetic ataxia.

d) Other TMS measures

(i) F-wave amplitudes in genetic ataxia

F wave amplitudes, elicted by a supramaximal stimulus to the ulnar nerve, were normal in SCA6, but significantly enlarged in SCA1, 2 and 3 and Friedreich's Ataxia.[219]

e) Summary of TMS alterations in genetic ataxia

TMS response in relation to the underlying genetic defect of ataxia syndromes (for example the subforms of the spinocerebellar ataxias) have been studied by some groups with a comprehensive study by Schwenkreis et al.[219] This revealed that changes of excitability are not generally present in genetic ataxias but are restricted to some subtypes. rTMS data in genetic ataxia are limited to one small pilot study.

Chapter 6 Methods

The experiments were approved by the ethics committee of the National Hospital and Institute of Neurology, London.

I. Subject Ascertainment

Patients with Huntington's disease and young-onset PD due to parkin mutations were ascertained from pre-existing databases of patients from the movement disorder clinics at the National Hospital for Neurology and Neurosurgery who tested positive for the IT15 and parkin mutation. Inclusion criteria were 1) genetic analysis positive for the IT15 or parkin mutation, 2) no brain, spinal or peripheral nerve surgery in the past, 5) no history of other neurological or psychiatric disease other than related to Huntington's disease, in particular, no history of epilepsy.

These subjects were contacted by telephone and the study was discussed with them. Subjects who expressed interest in the study received further details. Participating subjects were clinically examined and disease severity rated according to the Unified Parkinson's Disease Rating Scale (UPDRS) motor part III and the Unified Huntington's Disease Rating Scale (UHDRS) scale. Details on patients' age at onset, response to treatment, other medical history were recorded.

Family members without symptoms had previously given a blood sample for mutation analysis on the understanding that no results of the gene anaylsis would be made available to them. Subjects who wished to know their gene test result were refered for genetic counselling to the clinical genetics service at the National Hospital for Neurology: the process of counselling and delivery and

follow-up of these patients was therefore performed separate from the study as part of normal NHS clinical service provision. Subjects were informed that both mutation positive and negative subjects would be invited to take part in the elctrophbysiological studies, and therefore an invitation to take part should not be taken as evidence of mutation carriage.

In practice, many asymptomatic parkin and all presymptomatic HD gene mutation carriers knew their mutation status, which simplified the potential ethical dilemmas associated with this type of study.

1. Recruitment of HD Subjects

We recruited a total of 16 subjects with a molecular genetic diagnosis of Huntington's disease (for CAG repeat length see table 7.1 (3 male, 13 female). We also recruited 22 healthy control subjects (13 male, 9 female) with a mean age of 36.1 years (range 28-58). HD subjects were examined clinically; the UHDRS to score motor symptoms. Accordingly, eight patients were classified as pre-symptomatic that is they had a score of 7 or less on the UHDRS motor subscale as well as no psychiatric or cognitive symptoms. The remaining eight patients were classified as early symptomatic. The time to symptom onset was calculated according to Langbehn et al[225]. Clinical details of these patients are given in table 7.1. Patients and controls were not taking medication at the time of the study.

2. Recruitment of Parkin Subjects

We studied eight genetically proven parkin patients (5male :3 female). Of these, six were compound heterozygotes and two were homozygotes for the parkin mutation.

Seven genetically proven carriers (4 male :3 female), all parents of parkin patients were also studied. Five of these had participated in a previous 18F-dopa PET[35] study which had revealed mild dopaminergic deficits in the caudate or putamen in three of them. Characteristics of patients and carriers are summarized in Table 8.1.

Because of mean age differences between patients and carriers two different control groups were studied; one age-matched for the patients, the second age-matched for the carriers. No participant took CNS active drugs except dopaminergic medication which was withdrawn prior to assessment. Parkin patients were studied "off" medication, meaning they withdrew from their dopaminergic medication for 12 hours prior to TMS assessment. This is compatible with half lives of the medication these patients were on, which were combinations of trihexyphenidyl (half life 3-4 hours), levodopa (half life of standard preparation 50 minutes and 1.5 hours when taken with carbidopa) and ropinirole (half life 6 hours).

II. Electrophysiological Methods

Different electrophysiological techniques were used in the experiments:

TMS Experiment 1: TMS biomakers in pre-symptomatic HD subjects and early disease HD patients compared to healthy controls: assessment of thresholds, input/output curves, intracortical inhibition, silent period and short afferent inhibition. Results are described in Chapter 7

TMS Experiment 2: A TMS footprint in manifesting and non-manifesting parkin subjects compared to normal subjects. We assessed thresholds, input/output curves, silent period, central motor conduction time and intracortical inhibition and facilitation. Results are described in Chapter 8

These methods are described in turn below.

1. TMS methods used in Experiment 1 and Experiment 2

Subjects were seated in a comfortable chair with their eyes open. EMGs were recorded via Ag/AgCl electrodes placed over the first dorsal interosseous (FDI) using a belly-tendon montage. Signals were filtered (30Hz-10KHz), amplified (Digitimer 360, Digitimer Ltd., Welwyn Garden City, Herts, UK) and then stored on a computer via a Power 1401 data acquisition interface (Cambridge Electronic Design Ltd, Cambridge UK). Analysis was carried out using Signal Software (Cambridge Electronic Design).

TMS was delivered on the hemisphere contralateral to the most affected body side in Parkin patients and over the left hemisphere in HD patients and asymptomatic HD and Parkin carriers and healthy controls. Magnetic stimuli were given using a hand-held figure-of-eight coil with an (outer winding diameter 9 cm). For single-pulse TMS the coil was connected to a monophasic

stimulator. For paired-pulse paradigms two magnetic stimulators were connected using a Y-shaped cable (all Magstim Co, UK). The optimal spot ("motor hot spot") was defined as the location on the scalp where TMS resulted consistently in the largest Motor Evoked Potential (MEP). For active measures subjects were asked to maintained a steady background contraction of about 20% of the maximum, as assessed visually on an oscilloscope. The following measures were performed:

a) Motor thresholds (MT)

These were measured at rest (RMT) and during background voluntary contraction (AMT), using validated criteria.[226] MTs mainly reflect the excitability of axonal membranes.

Resting motor threshold (RMT) was defined as the minimum intensity needed to evoke an MEP of >50μV in 5 out of 10 consecutive trials in the relaxed FDI. Active motor threshold (AMT) was defined as the minimum intensity (in % of maximum stimulator output) needed to evoke a MEP of >200μV in 5 out of 10 trials in the tonically active FDI (~20% of maximal contraction as assessed visually on an oscilloscope). Thresholds were approached from above threshold in steps of 1% stimulator output. Once no MEPs could be elicited the intensity was increased in steps of 1% stimulator output until a minimal MEP was observed. This intensity was taken as motor threshold.

b) Input/Output Curves of MEP amplitude

During background contraction, 10 MEPs were collected at each stimulation intensity starting with 100%RMT and increasing stepwise by 10%RMT in the

Parkin experiment and by 25%RMT in the HD experiment up to 150%RMT of the stimulator's maximal output while the patients were maintaining a background contraction of 20% of their maximum power. Ten trials were recorded, and the average MEP area was taken as MEP size. For amplitude measurements peak-to-peak values were averaged.

Recruitment of MEP amplitude, especially in the active state, assesses the amount of corticospinal output available to TMS. The gradient of the curve provides information regarding the distribution of excitability within the whole motor neuronal pool.

c) Central Motor Conduction Time during background contraction

In the Parkin experiment, central motor conduction time (CMCT) was assessed. Based on the recordings of the MEP recruitment curves, CMCT was calculated as follows: The maximum peripheral delay was deducted from the minimum MEP latency at each stimulation intensity. To calculate the peripheral delay spinal roots (C6/C7) were stimulated at 150%RMT. CMCT measures provide information on the velocity of conduction within the corticospinal tract.

d) Silent Period

As described above, the cortical silent period (CSP) is a period of EMG silence that occurs in a voluntarily contracted muscle following a suprathreshold magnetic stimulation given over the contralateral representative motor area. CSP is thought a measure of cortical inhibitory mechanisms that operate during voluntary contraction, possibly mediated by $GABA_B$-ergic circuits. In normal

subjects duration of CSP is typically 120ms, although this can be longer if the stimulation intensity is increased.[95]

In Parkin patients, CSP duration was measured at 130 and 140% RMT from the onset of the active MEP to the reappearance of uninterrupted EMG activity of a pre-stimulus mean amplitude. In HD patients, CSP duration was measured at 130, 150 and 175% AMT.

The CSP/MEP AMPLITUDE ratio was calculated which is thought superior to absolute measures of CSP duration, because it does not depend on parameters like coil orientation and the stimulation intensity [92] and the ratio represents an additional measure of the inhibitory circuits underlying the CSP. In HD subjects, the area under the MEP was also determined.

e) Intracortical Inhibition and Facilitation.

Ten paired-stimuli were delivered randomly at 2, 3, 7, 10 and 15 ms and intermixed with ten single test stimuli. In parkin studies, the conditioning stimulus (CS) was set at 80%AMT and the test stimulus (TS) at an intensity that produced MEPs of about 1mV. Short interstimulus intervals typically result in inhibition (SICI) and longer intervals in facilitation (ICF). Data from 2 and 3ms and from 7, 10 and 15ms were therefore pooled and averaged to calculate the amount of SICI and ICF respectively. SICI and ICF were expressed as the ratio between conditioned and unconditioned trials. In HD subjects, ICF was not assessed.

f) Recruitment Curve of SICI

In Parkin subjects, SICI patterns were more thoroughly assessed by recruitment curves. Paired stimuli were delivered randomly at different conditioning

stimulation (CS) intensities (70, 80, 90 or 100% of AMT) intermixed with single test stimuli (10 trials per condition). The test stimulus intensity was set to produce MEPs of 1mV. These measures provide further information on the excitability properties of the inhibitory interneurons by assessing the change in the amount of inhibition in respect to the CS intensity. Deepest inhibition usually occurs at 80-90%AMT CS.

g) Short latency afferent inhibition by somatosensory input from the median nerve

In HD subjects, we assessed short latency afferent inhibition of the motor cortex as previously described[103]. A test MEP of ~1mV peak-to-peak amplitude was elicited in the FDI by TMS. A paired pulse paradigm examined the influence on MEP size of a supra-threshold electrical stimulus given to the median nerve through bipolar electrodes. The electrical stimulus to the median nerve was delivered at an intensity just above the threshold to elicit a visible contraction in the thenar muscles and preceded the TMS pulse to the FDI hot spot by 14, 18, 20, 22, 24, 26 or 29ms. Twenty trials of the MEP elicited by TMS alone and 10 trials of conditioned MEPs for each ISI were collected. The amplitude of the MEP in the FDI was measured with in-house software. The average amplitude of the conditioned MEP was expressed in percent of the average amplitude of the test MEP alone.

2. Statistical Analysis

Data were collected without knowledge about the clinical status or disease severity of patients. Peak to peak amplitude of MEP, the area under the curve of

the MEP and the silent period duration were measured with in-house software. All statistical analyses were performed using SPSS 11 for Windows software package. Statistical significance levels were set to p=0.05. Statistical differences in the ANOVAs were followed by a post-hoc paired t-test analysis. Mauchly's test was used to test for sphericity in the repeated measures ANOVAs, and the Greenhouse-Geisser correction was applied to the DFs if necessary. Correlations of the CMCT were performed using non-parametric tests (Spearmann's rho correlation coefficient).

Parkin patients and carriers were always compared to their corresponding age-matched control group (referred to as "controls" only). When both control groups were compared they are referred to as younger controls and older controls.

The slopes of I/O curves at rest and during activity, the silent period recruitment and SICI recruitment were fitted with linear regression in each subject. Two factor ANOVA was used to compare groups with GROUP as in-between-subject factor. The second factor was stimulation intensity with the different levels as applicable (e.g. 100, 110, 120, 125, 130, 140 and 150%RMT for RC_{MEP} and RC_{CMCT}; 130% and 140%, 150% and 175% for CSP; 70, 80, 90 and 100%AMT for RC_{SICI} at 2ms). Post-hoc tests with Bonferonni correction were used when indicated. For CMCT, 95% confidence intervals (95%CI) of the CMCT distribution were calculated in controls and CMCT in patients was stratified as normal (within the 95%CI) or abnormal (outside the 95%CI).

Similarly, for paired pulse paradigms repeated-measures analysis of variance (ANOVA) was used to assessd ICI and ICF and whether there was a main effect of ISI on MEP size or conditioning stimulus intensity (60, 70, 80, 90, 100%AMT) on the amount of SICI; or an effect of ISI on MEP size in the short-

latency afferent inhibition paradigm. Because inhibition and facilitation at particular interstimulus intervals have different mechanisms, we grouped means at an "inhibitory" interval (average of 2, 3, and 4ms interstimulus intervals), and a "facilitatory" interval (average of 7, 10 and 15ms interstimulus intervals).

In order to assess how TMS parameters (RMT, SICI threshold, slopes of the I/O curve for MEP size at rest, maximum SAI) were associated with the estimate of time to onset of symptoms, or the UHDRS/UPDRS motor score, we used backward stepwise regression analysis with 'years to onset' or 'motor score' as the dependent variable. In the HD experiment, we entered 'maximum SAI', 'CAG repeat length' and 'age' as independent parameters. A parameter was removed from the model if the probability of its contribution was less then 0.1. MTs and age were compared using independent samples t-tests.

Chapter 7 Results in HD Subjects

Details of the HD participants including demographic, molecular genetic and clinical data are given in Table 7.1.

Patient	age	gender	CAG	UHDRS motor	Years to predicted onset
Premanifest 1	41	F	43	0	10.59
Premanifest 2	39	F	42	2	16.10
Premanifest 3	40	M	41	4	19.10
Premanifest 4	32	M	40	0	33.54
Premanifest 5	28	F	47	7	10.50
Premanifest 6	38	F	40	2	27.68
Premanifest 7	38	F	40	1	27.68
Premanifest 8	53	F	41	5	9.44
Manifest 1	44	F	43	8	
Manifest 2	48	M	47	16	
Manifest 3	43	F	43	15	
Manifest 4	33	F	44	13	
Manifest 5	48	F	46	23	
Manifest 6	37	F	43	8	
Manifest 7	64	F	44	30	
Manifest 8	40	F	46	30	

Table 7.1: Demographic, molecular genetic and clinical data from HD patients. Predicted symptom onset was calculated according to Langbehn et al.[225] All premanifest patients had a diagnostic confidence score of less than 4 on the UHDRS.

There was no significant difference in the mean age of HD subjects (41.6 years, range 28-64) and control subjects (36.1 years, range 28-58).

I. Motor thresholds and motor cortex excitability at rest

Resting motor thresholds of all patients taken together (mean 43.4, SE 1.7, 95%CI 40-46.8) were higher than in controls (mean 38, SE 1.4, 95%CI 35.5-41) (ANOVA, main effect of 'group', $F_{1,36}=5.57$, $p=0.024$).

Dividing patients into the subgroups of premanifest (mean 42.6, SE 2.4, 95%CI 37.8-47.5) and early manifest patients (mean 44.1, SE 2.4, 95%CI 39.3-49) there was still a trend towards higher RMT in patients than in controls (ANOVA, main effect of 'subgroup', $p=0.07$).

Thresholds with pre-activation (AMT) were also higher in patients (mean 33.2, SE 1.5 95%CI 30.1-36.2) than in controls (mean 28, SE 1.2, 95%CI 25.5-30.5) (ANOVA, main effect of 'group', $F_{1,36}=7.04$ $p=0.012$) even if dividing patients into premanifest (mean 32.8, SE 2.2, 95%CI 28.4-37.1) and early manifest (mean 33.6, SE 2.2, 95%CI 29.3-38) (ANOVA, main effect of 'subgroup', F2,36=4.74, $p=0.042$). Pairwise comparison revealed that the main difference was between controls and early manifest patients ($p=0.03$) with a trend comparing controls and premanifest patients ($p=0.065$).

II. Input/Output Curve

Above RMT, MEP size increased with increasing stimulation intensity (repeated measures ANOVA, main effect of 'stimulation intensity', $F_{2,72}=55.4$, $p<0.0001$). However, patients recruitment slopes were flatter than those of controls (repeated

measures ANOVA, interaction 'intensity*group', $F_{2,72}$=7.9, p=0.001) even with two patients subgroups (repeated measures ANOVA, interaction 'intensity*group', $F_{2,72}$=3.9, p=0.006, Figure 7.1A). Post-hoc pairwise comparisons showed that the slope of both premanifest (0=0.013) and early manifest patients (p=0.017) was flatter than in controls (Figure 7.1A) whereas the slopes of both patient subgroups were similar.

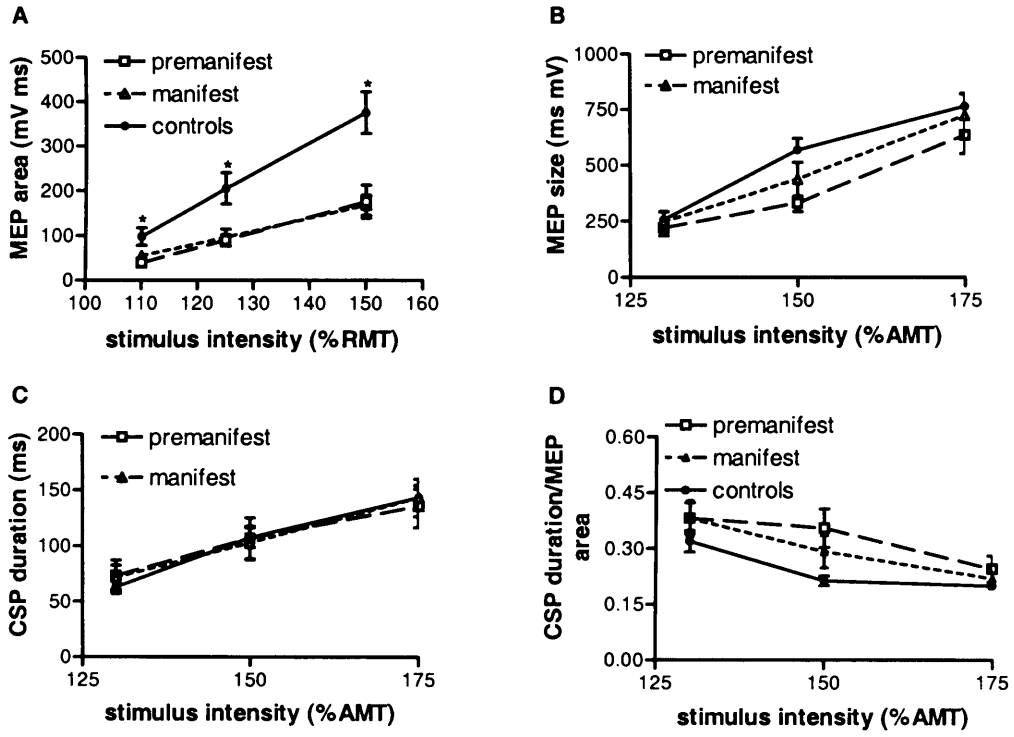

Figure 7.1: cortico-spinal system excitability. **A.** MEP size recorded from relaxed FDI after TMS shock to the M1 hand area with 110%, 125% or 150% of RMT. Patients recruitment slopes were flatter than those of controls (repeated measures ANOVA, interaction 'intensity*group', $F_{2,72}$=3.9, p=0.006). Post-hoc pairwise comparisons showed that the slope of both premanifest (0=0.013) and early manifest patients (p=0.017) was

flatter than in controls whereas the slopes of both patient subgroups were similar. **B-D.** MEP size and CSP duration recorded from active FDI after TMS shock to the M1 hand area with 130%, 150% or 175% of AMT. MEP area (**B**), CSP duration (**C**) and the ratio of CSP duration and MEP area (**D**) are similar in controls and HD patients. Values are means ±SEM, n=16 for HD patients (n=8 premanifest, n=8 early manifest), n=22 for controls.

III. Motor cortex excitability with pre-activation and cortical silent periods

MEP size (area) increased significantly with increasing stimulation intensity in both the controls and the patients (repeated measures ANOVA, $F_{2,70}$=97.76, $p<0.001$, Figure 7.1B). This increase in MEP size was similar in patients and controls. Cortical silent period duration also increased with increasing stimulation intensity (repeated measures ANOVA, main effect of 'stimulation intensity', $F_{2,70}$=136.3, $p<0.001$, Figure 7.1C) without major differences between patients and controls. The same was true for the ratios of cortical silent period duration and MEP area (Figure 7.1D).

IV. Short interval intra-cortical inhibition

We distinguished between the threshold intensity needed to produce SICI and the amount of SICI at suprathreshold intensities of conditioning shock..[227]

First we determined the threshold for SICI as described previously[227] which for technical reasons was not possible in one control and in two premanifest patients.

SICI thresholds were lower in controls (mean 17.9%, SE 0.79, 95%CI 16.25-19.49) than in patients (mean 20.8, SE 1, 95%CI 18.7-22.9) (ANOVA, main effect of 'group', $F_{2,34}$=5, p=0.032). This effect was lost when patients were divided into the premanifest and early manifest subgroups.

Above threshold increasing the conditioning stimulus intensities reduced conditioned MEP size (repeated measures ANOVA, main effect of 'conditioning stimulus intensity', $F_{2.56, 95.8}$=90.15, p<0.001, Figure 7.2). The recruitment slope was similar in patients and controls (repeated measures ANOVA, no significant interaction of 'group*intensity').

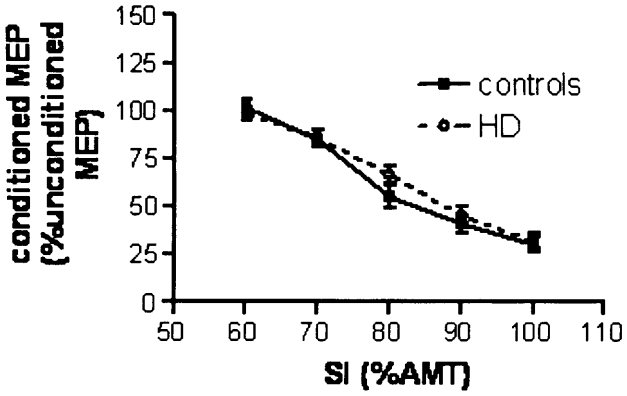

Figure 7.2: Short intracortical inhibition (SICI). In controls and patients increasing intensity of the conditioning stimulus reduced the size of the conditioned MEP in a similar way. Values are means ±SEM, n=14 for HD patients, n=22 for controls.

V. Short latency afferent inhibition

In controls and patients, a supra-threshold electrical stimulus to the median nerve at the wrist before the TMS pulse to the FDI hot-spot reduced the mean amplitude of the test stimulus predominantly at ISIs of 20, 22 and 24ms (repeated measures ANOVA, main effect of 'ISI', $F_{2.34,84.31}=17.28$, $p<0.001$, Figure 7.3).

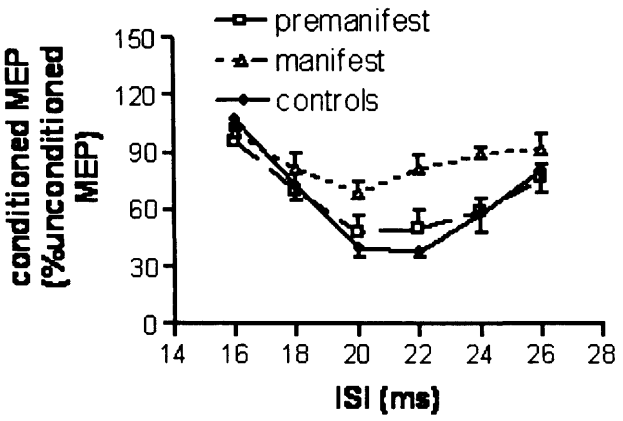

Figure 7.3: Short latency afferent inhibition (SAI). The SAI curve was flatter for manifest HD patients compared with controls or premanifest patients.

Since the early period of inhibition is more likely to have a partly cortical origin than later timings[103] we assessed the maximum amount of afferent inhibition in each individual. Maximal SAI was greatest in controls followed by premanifest patients and early manifest patients (ANOVA, main effect of 'group', $F_{2,35}=19.7$, $p<0.001$, Figure 7.4). Post-hoc pairwise comparisons revealed that early manifest patients (mean 68.8, SE 5.5, 95%CI 57.6-80) differed from controls (mean 28.5, SE 3.3, 95%CI 21.8-35.3, $p<0.001$) and premanifest patients (mean 37.15, SE

5.5, 95%CI 26-48.3, p<0.001, Figure 7.4). Premanifest patients and controls were similar.

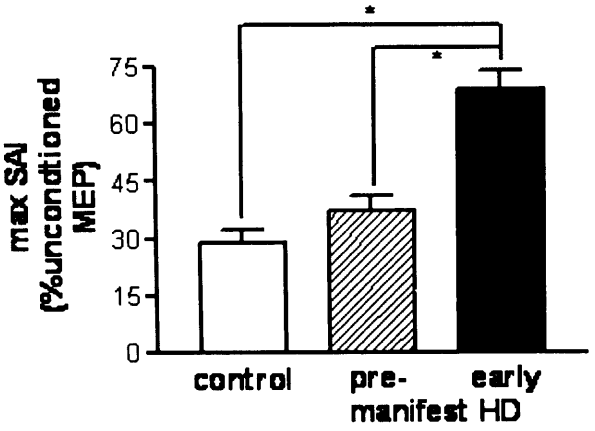

Figure 7.4. Maximal SAI was greatest in controls followed by premanifest patients and early manifest patients (ANOVA, main effect of 'group', $F_{2,35}$=19.7, p<0.001). Post-hoc pairwise comparisons revealed that controls and premanifest patients had more SAI than early manifest patients (*p<0.001). Values are means ±SEM, n=16 for HD patients (n=8 premanifest, n=8 early manifest), n=22 for controls.

VI. Correlation of electrophysiological parameters and clinical measures

We examined whether any of the electrophysiological parameters were associated with the presumed disease state. Only maximum SAI served as a predictor for estimated years to motor onset (backward stepwise regression analysis with 'years to onset' as dependent variable, ANOVA, $F_{1,13}$=8.2, p=0.013, R=0.61, R^2=0.37, adjusted R^2=0.32, Figure 7.5).

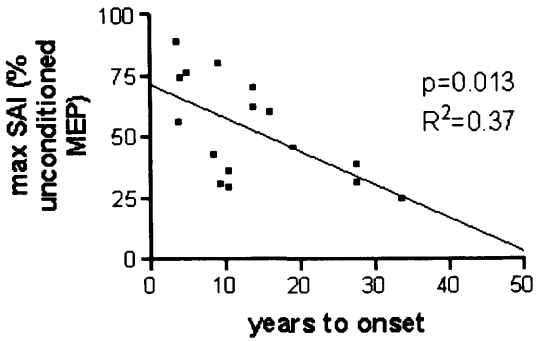

Figure 7.5: Backward stepwise regression analysis. There was a significant correlation of max SAI with predicted years to onset of symptoms. Data are from 16 Huntington patients.

We then correlated clinical severity (UHDRS motor score) with electrophysiological parameters.

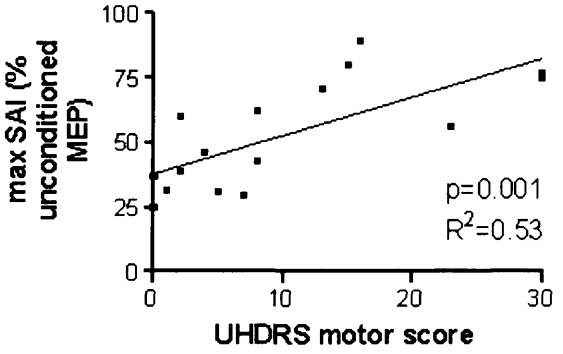

Figure 7.6 Backward stepwise regression analysis. There was a significant correlation of max SAI with the UHDRS motor score. Data are from 16 Huntington patients.

Again, maximum SAI was the only predictor of UHDRS motor score (backward stepwise regression analysis with 'UHDRS motor score' as dependent variable,

ANOVA, $F_{1,13}$=15.63, p=0.001, R=0.73, R^2=0.53, adjusted R^2=0.5, Figure 7.6). Next, we examined for correlations of clinical severity with CAG repeat length, age and maximum SAI. This model strongly predicted the UHDRS motor score (ANOVA, $F_{3,12}$=11.4, p=0.001, R=0.86, R^2=0.74, adjusted R^2=0.68).

Chapter 8 Results in Parkin subjects

Patients were significantly younger than carriers (p=0.003); their UPDRS motor score was 35.3±11 (table 8.1). Four had hyperreflexia clinically. 11 healthy individuals (mean age 46.8±11.5 years) formed the "younger control" group; the group of "older controls" comprised of 10 healthy individuals (mean age 69.2±5 years).

Clinical Phenotype	Subject	Age	Age at onset	UPDRS score	Hyper-reflexia	18F-Dopa influx, Ki (min^{-1}), in caudate; putamen
Manifesting parkin patients	1/ M **	33	13	37	+	see Khan et al.[35] for group results
	2/M *	37	10-15	22	--	
	3/M *	57	22	27	--	
	4/F **	63	39	39	+	
	5/F *	38	19	42	+	
	6/M *	48	34	37	--	
	7/M *	46	29	22	--	
	8/F *	66	55	27	+	
Asymptomatic parkin heterozygotes	1/M *	73	--	0	--	0.0146; 0.0136
	2/M *	63	--	0	--	0.0122; 0.0121#
	3/F *	65	--	0	--	0.0094##; 0.0133
	4/M *	64	--	0	--	0.0108##; 0.0126#
	5/F *	69	--	0	--	0.0154; 0.0179
	6/M *	66	--	0	--	n.d.
	7/F *	71	--	0	--	n.d.
Means						
Parkin patients		48.5±12.		35.3±11		
Parkin carriers		66.5±3.4		0		
Young controls (n=11)		46.8±11.5		0		
Old controls (n=10)		69.2±5		0		

Table 8.1. Demographic and clinical characteristics of patients and asymptomatic parkin heterozygotes (carriers). * heterozygote for parkin gene mutation; ** homozygote for parkin gene mutation. # 1.5 SD below normals; ## 2 SD below normals; n.d. no data

I. Resting and Active Motor thresholds

Motor thresholds were the same in patients and controls (RMT: 40±6 vs 40±10, p=0.9, AMT: 34±5 vs 32±7, p=0.5) and between the two control groups. (Figure 8.1). Carriers had significantly higher RMT (51±9 vs 40±7, p=0.015) and AMT (42±98 vs 32±4, p=0.03) than controls.

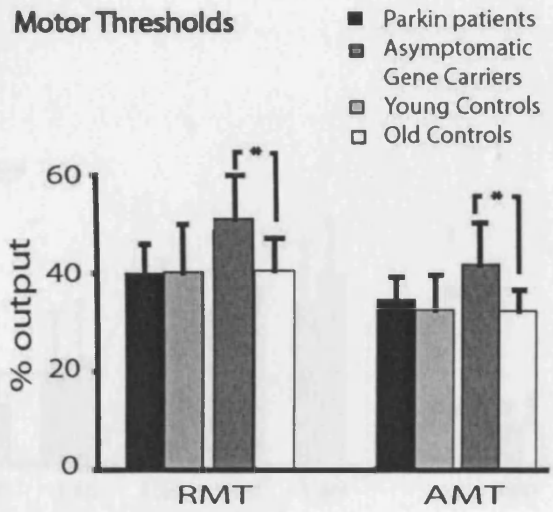

Figure 8.1 Resting and active motor thresholds for patients, carriers and control subjects. There was no difference between patients and their age-matched controls. Carriers had significantly higher RMT and AMTs compared to their controls. The two control groups were not different. *= significantly different from the corresponding age-matched group ($p<0.05$); RMT: resting motor threshold; AMT: active motor threshold.

II. Input/output curve

The inout/output curve during background contraction is demonstrated in Figure 8.2. A two-factor ANOVA between patients and controls showed a significant effect of SI ($F(4,68)=56.2$, $p<0.001$) but not GROUP with no SI *X* GROUP interaction. Comparisons between carriers and controls as well as between younger and older controls yielded similar results. This indicates that all the groups behaved similarly with increasing intensities of TMS.

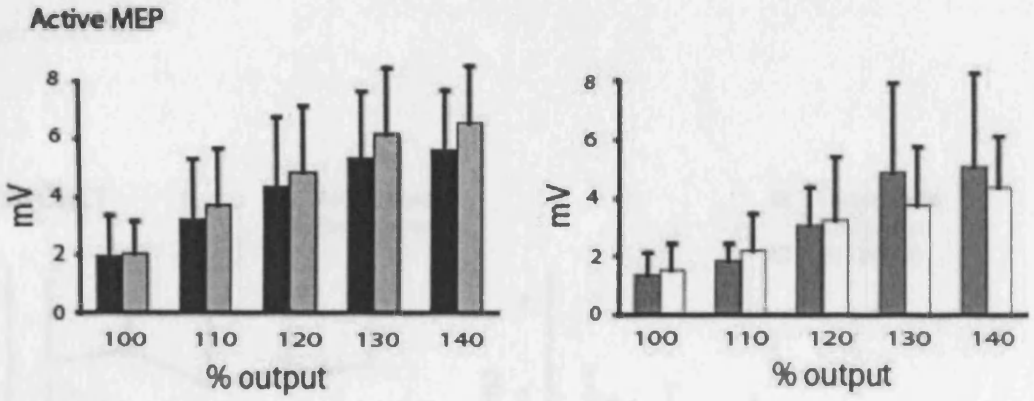

Figure 8.2: Recruitment of active MEPs. There were no significant differences in the recruitment of the active MEPs between patients and their controls or carriers and their control group. In all groups, the amplitude of the active MEPs was significantly increased with higher stimulation intensity.

III. CMCT

CMCT was significantly longer in patients compared to controls. (Figure 8.3) A two-factor ANOVA showed a significant main effect of SI ($F(4,56) = 11$,

p<0.001), a significant SI *X* GROUP interaction (F(4,56)=5.7, p=0.007); and a non-significant effect of GROUP (F(1,14) = 4, p=0.063). Subsequent one-factor ANOVA showed a significant main effect of SI in the controls (F(4,36) = 29.3, p<0.001); CMCT significantly decreased as intensity increased and in most subjects reached a plateau at 120%RMT. In the patients there was no main effect of SI; i.e CMCTs did not change with increasing SI; pair-wise comparisons revealed that patients had significantly higher CMCTs compared to controls at 110%RMT (p=0.03), 130%RMT(p=0.006) and 140%RMT (p=0.01).

CMCTs were similar between carriers and controls and between older and younger controls.

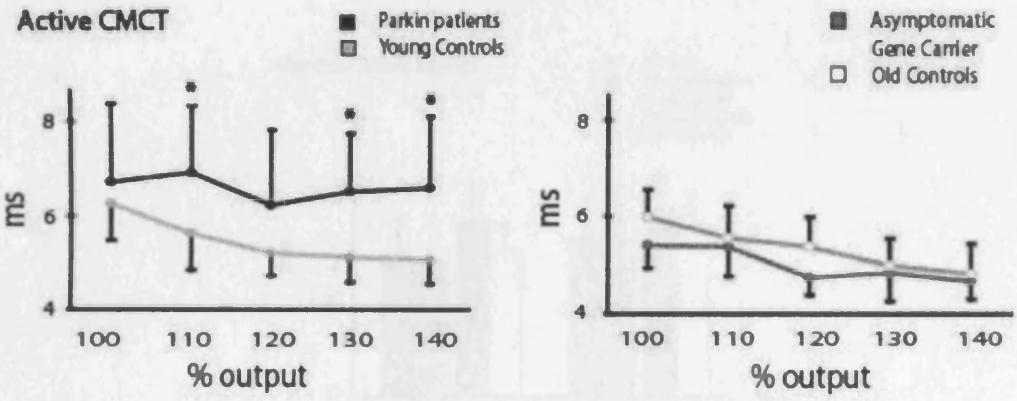

Figure 8.3. Recruitment of active central conduction time (CMCT): Patients had significantly longer CMCT in actively contracting muscles compared to their age-matched controls at 110%RMT, 130%RMT and 140%RMT. Carriers had similar CMCTs to their controls. The two control groups were not different from each other. In the carriers and the two control groups CMCT was significantly shorter with increasing stimulation

intensity. This was not seen in the patients. * = significantly different among groups ($p<0.05$)

IV. CSP duration

The CSP duration is shown in Figure 8.4. Two factor ANOVA on CSP duration showed only a significant main effect of SI for all comparisons (patients vs controls, carriers vs controls and between the two control groups). That indicates that all groups behaved similarly when the intensity was changed. When we used the CSP/MEP AMPLITUDE ratio for the analysis, again there was no difference between groups for all comparisons.

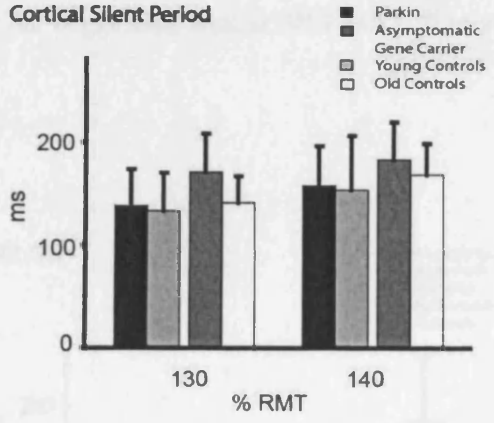

Figure 8.4. Absolute duration of the cortical silent period for patients, carriers and both control groups. There were no significant differences between groups or within groups with increasing intensity. RMT: Resting motor threshold.

V. SICI /ICF

Rsults for SICI and ICF in Parkin patients and carriers compared to controls are depicted in Figure 8.5. A two-factor ANOVA between patients and controls revealed a significant main effect of ISI ($F(1,15)=83.5$, $p<0.001$) with no effect of GROUP and no ISI *X* GROUP interaction; thus, both groups showed similar values for SICI and ICF.

Comparing carriers and controls, ANOVA showed a significant main effect of ISI ($F(1,14)=49.8$, $p<0.001$) and a significant main effect of GROUP ($F(1,14)=14.7$, $p=0.002$) but no ISI *X* GROUP interaction; subsequent t-tests showed that the GROUP effect was due to a non-significant tendency in carriers towards reduced SICI ($p=0.06$). Finally, comparison between the two control groups showed a significant effect of SI ($F(1,17)=94.04$, $p<0.001$), but no effect of group or an interaction; suggesting similar SICI and ICF in younger and older controls.

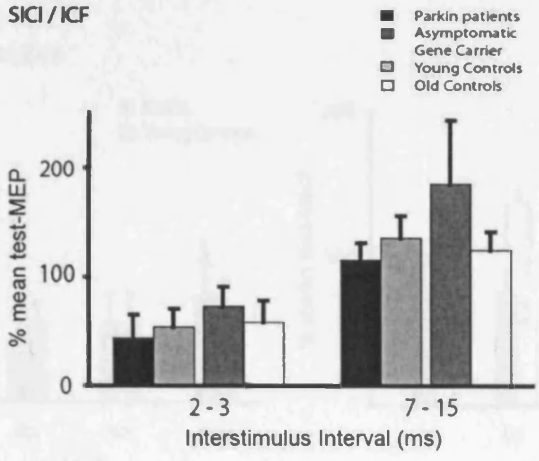

Figure 8.5. Short interval intracortical inhibition (SICI) and intracortical facilitation (ICF). SICI /ICF are expressed as percentage change from the mean test-MEP.

Patients and their controls had similar SICI and ICF; carriers tended to have reduced SICI compared to their control group, but this difference was not significant(p=0.06). There was no difference between the two control groups.

1. SICI Recruitment Curve

The SICI recruitment curve at 2ms is shown in Figure 8.6. A two-factor ANOVA between patients and controls showed a significant main effect of SI ($F(3,45)=6.5$, $p=0.004$), but no effect of GROUP, nor a SI X GROUP interaction; subsequent one factor ANOVAs verified a main effect of SI in patients ($F(3,18)=5.4$, $p=0.008$) and controls ($F(3,27)=3.7$, $p=0.02$). This suggests normal excitability in inhibitory circuits in parkin patients.

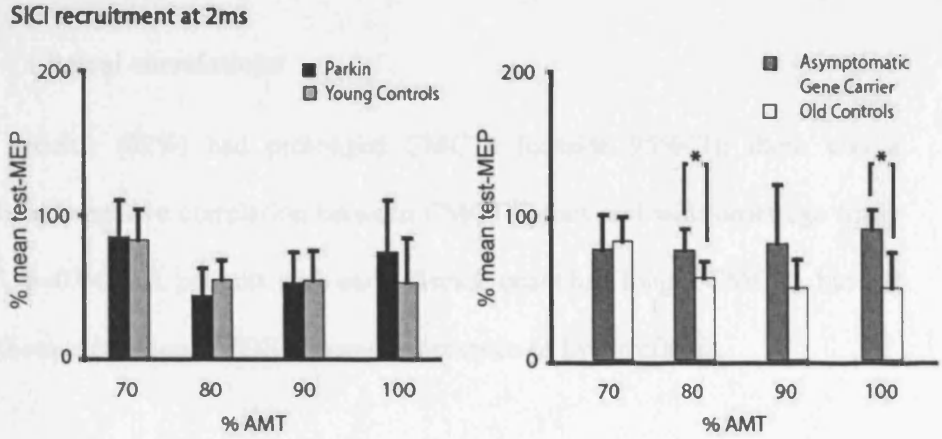

Figure 8.6. Short interval intracortical inhibition (SICI) recruitment curve at 2ms for patients, carriers and the two control groups. Patients and their

controls had similar SICI: controls showed significantly stronger SICI at 80%AMT and 90%AMT; in patients SICI was consistently stronger at 80%AMT but not at higher stimulation intensities.

In the carriers SICI fail to increase with increasing stimulation intensity. Compared to their controls there was a significantly reduced SICI at 80%AMT and 100%AMT. There was no difference between the two control groups. * = significant difference between groups. ($p<0.05$)

Comparing carriers to controls, two factor ANOVA showed no effect of SI, but a significant effect of GROUP ($F(1,13)=6.4$, $p=0.025$) together with a significant SI X GROUP interaction ($F(3,39)=4.4$, $p=0.01$). Subsequent one-factor ANOVAs revealed that there was no main SI effect in the carriers, i.e. SICI did not change with increasing stimulation intensity; compared to aged-matched controls carriers there was less inhibition at 80%AMT ($p=0.015$) and 100%AMT ($p=0.02$). Finally, comparison between both control groups showed that both control groups had similar SICI recruitment.

VI. Clinical correlations

Five patients (62%) had prolonged CMCTs (outside 95%CI); there was a significant negative correlation between CMCT values and with onset age ($rho= - 0.83$, $p=0.04$), i.e. patients with early disease onset had longer CMCTs, but not with disease duration, UPDRS scores or presence of hyperreflexia.

Chapter 9 Discussion

Here we used TMS to ascertain an electrophysiological footprint of patients with genetic movement disorders.

I. Discussion of the findings in HD subjects

In the experiments we showed that patients with HD, both premanifest and early manifest, have higher motor cortex thresholds both at rest and in a pre-activated state. SAI, a measure of sensory-motor integration, is reduced in early manifest patients compared with controls and premanifest patients. In addition, SAI is inversely associated with predicted years to onset of HD signs and the UHDRS motor score, and a combination of age, CAG repeat length, and SAI strongly predicted the UHDRS motor score.

Threshold measures depend on the excitability of axon membranes at the site of stimulation and the membrane potential of postsynaptic neurones in motor cortex and spinal cord. If the latter is depolarised then excitatory inputs are more likely to cause the postsynaptic cell to discharge than if the membrane potential is hyperpolarised. During active contraction, synaptic excitability is high so that changes in threshold usually are thought to reflect changes in axonal excitability. The fact that active threshold was higher in HD thus suggests that axonal excitability was reduced. At rest, threshold will also depend on postsynaptic membrane potential. Whether this additionally contributes to reduced resting thresholds in HD is uncertain, although the reduced slope of the resting recruitment curve would be compatible with additional synaptic effects. However, these electrophysiological parameters were not associated with the severity of patients' motor signs. This suggests that motoneurones and their

modulation by inhibitory inter-neurones, i.e. the quality and shaping of the motor command, may not necessarily change as HD advances from the premanifest to the early manifest stage.

In contrast to these threshold changes, the electrophysiological measure of inhibitory interactions of sensory input and motor output, SAI, was related to clinical signs. It was reduced in early manifest patients but not in premanifest patients and showed an inverse relationship to UHDRS motor scores. Effectively, this means that once the balance of sensory input and motor output is disturbed, patients develop motor signs. Whether this is a causal connection or simply an association is unknown. However, the abnormalities in sensory-motor interaction indicated by abnormal SAI may well be associated with the known reduction in amplitude of somatosensory evoked potentials in manifesting individuals. [133]

Changes in the basal ganglia and various cortical areas occur before symptom onset in HD. These include the formation of neuropil aggregates,[228] oligodendrocytes,[229] and imaging abnormalities[230,231] including focal cortical thinning and pyramidal tract white matter abnormalities.[232,233] Our results suggest that increases in axonal thresholds could be added to this list, and may also be an intrinsic reaction of the brain to the presence of the mutated huntingtin protein.

Mutated huntingtin probably confers not only a toxic gain of function but also a loss of function.[234] Huntingtin plays an important role in neuronal development[235-237] and life-long expression of mutant huntingtin may give rise to inherent abnormalities in the development of the HD brain. Our results of abnormal thresholds could alternatively reflect carrying the HD mutation irrespective of whether the patient has motor manifestations rather than a result of a dynamic pathological process of HD.

In contrast, our measure of sensory-motor integration, SAI, was normal until patients developed symptoms. It changed gradually over time such that together with the patient's age, and CAG repeat length the level of SAI predicted symptom severity indicating this reflects the disease process rather than the HD trait. The SAI paradigm used in our study very likely involves cholinergic trans-synaptic pathways.[238,103] Cholinergic abnormalities have been described in post-mortem striatal tissue in HD[239] and transgenic mice. [240] The striatum and cortex degenerate most in the course of HD.[241,242] Recent evidence indicates cortical cholinergic changes precede those in the striatum.[243] In premanifest patients this includes the pre-frontal cortex, an area relevant for sensory-motor integration.[244] Thus our data suggest a continuous decline of cholinergic function in sensory inputs as the disease progresses similar to Alzheimer's disease.[245] In Alzheimer's dementia SAI is also abnormal; interestingly in contrast to dementia with Lewy bodies where pathological changes are that of cortical Lewy bodies. A functional decline in cortical cholinergic function may be due to a loss of cholinergic synapses without neuronal cell loss[243,246] and contribute to cognitive symptoms in HD patients and animals before motor onset.[243] Cholinergic changes may be restricted to the cortex because the nucleus basalis of Meynert as the provider of most of the cholinergic cortical input does not degenerate in HD.[247] These thoughts have also been outlined in the corresponding publication.

II. Discussion of the findings in Parkin subjects

Parkin patients had prolonged CMCT, but normal motor thresholds and cortical inhibitory activity. Asymptomatic carriers had increased motor thresholds but

CMCT was normal. Additionally, carriers showed abnormalities in SICI recruitment suggestive of changes in the excitability of the inhibitory circuits not seen in patients.

The increased CMCT in parkin patients confirms the report by De Rosa and colleagues[130] in a smaller cohort of four parkin patients at a single stimulation intensity. Speculative causes for prolonged CMCT include demyelination of the corticospinal tract, loss of the largest fast conducting axons in the tract, or excessive synaptic delays at a cortical level in activating corticospinal fibers after the TMS pulse is given. Demyelination seems unlikely given MRI spine scans were normal and demyelination should lead to greater increases of CMCT than the 2ms seen here as well as smaller MEPs due to dispersion of impulses. De Rosa and colleagues did report decreased MEP amplitude in one of their patients, but our results in a larger number of patients do not confirm this. CMCT prolongation therefore may reflect changes in neuronal properties of the pyramidal cells themselves and/or their excitatory interneuronal input in the cortex usually activated by TMS (I-wave inputs). In control subjects and carriers, increasing SI led to a decrease in CMCT. This effect, usually ascribed to direct activation of pyramidal axons at high TMS intensities (D-waves), rather than the usual indirect activation that occurs at lower intensities (I-waves),[248,249] was not seen in patients. The combination of normal motor thresholds and normal MEP amplitude suggests that CMCT prolongation cannot be solely explained by I-wave recruitment abnormalities, although this cannot be excluded without I-wave facilitation studies. It seems more likely that pyramidal neuronal excitability at high stimulation intensities (D-wave recruitment) is abnormal.

CMCT was normal in carriers who in contrast to patients had increased thresholds. Thresholds are usually normal in PD and, if altered, they tend to be low.[129] Thresholds were also normal in the study by Baumer et al.[131]; however their cohort was on average twenty years younger than ours. (66.5±3.4 years in our study vs 40±1.2 in the study by [131]) It is possible that these younger carriers may have higher thresholds were they to be tested twenty years later. Our finding would be compatible with the idea that it reflects mild abnormalities in I-wave recruitment at low stimulation intensities. The different manifestations in patients and carriers could reflect the different disease load over time.

CMCT is normal in patients with idiopathic PD; abnormally prolonged CMCT could question the diagnosis of idiopathic PD [250]. Thus, CMCT measures may represent an additional tool for the identification of parkin disease. Prolonged CMCT here may be associated with the hyperreflexia commonly seen in parkin patients. Hyperreflexia is also common in MSA patients who also show abnormal CMCTs. [250] In this study we found no correlation between the presence of hyperreflexia and CMCT prolongation but this may be due to the small sample size. The significant relation with onset age may reflect pathophysiology but also requires confirmation with larger group sizes.

Our results for SICI and CSP suggest normal intracortical inhibitory activity in parkin patients. CSP, and more consistently SICI, have been found reduced in idiopathic PD, especially when studied off medication. However the finding is not specific since it occurs in several other movement disorders[20-25,17927,31-33], and also does not correlate with disease duration or severity. [251] The normal measures in Parkin may reflect different underlying pathomechanisms and earlier disease onset but whether the physiological profile is specific for parkin associated

parkinsonism or an epiphenomenon of Mendelian forms of parkinsonism is unknown.

Like a previous study in carriers,[131] we did not find significant abnormalities in SICI . However, compared to age-matched controls, carriers showed reduced SICI recruitment with increasing stimulation intensity. Normally, SICI becomes evident at conditioning intensities around 70%AMT and deepens gradually as the intensity rises to 80-100%, after which it begins to turns to facilitation.[97] It is important to note that our carriers also had significantly higher AMT. If we hypothesize that the threshold of the SICI interneurons does not increase in parallel with that of the interneurons mediating I waves (AMT), the SICI curve would move to the right, and intensities of 90% or 100% AMT would fall outside the range of inhibitory conditioning intensities for SICI. This is well described for stroke and studies have explored increased thresholds[252] and SICI curves using absolute rather than relative stimulation intensities. In our study SICI was significantly reduced on both ends of the curve suggesting that SICI recruitment abnormalities in carriers may not be just due to threshold differences.

One possibility is that SICI reduction represents some form of motor cortex adaptation to compensate for the mild (subclinical) dopaminergic dysfunction in carriers. In a recent functional imaging study,[253] changes in brain activation associated with self selected finger movements were interpreted as adaptation allowing carriers to maintain motor function despite subclinical dopaminergic deficits. A similar explanation was proposed for short afferent inhibition alterations seen in the carriers.[131] Such adaptations presumably require synaptic modifications in the cortex, mainly in the form of long term potentiation/depression (LTP/LTD), and may involve modulation of activity in

GABAergic inhibitory pathways. SICI is thought to be GABA-dependent. Finally, such compensatory mechanisms may cease to operate when the underlying deficit exceeds a certain threshold and overt clinical symptoms appear. That could explain why SICI abnormalities were not present in our patients. One way to test this hypothesis would be to investigate the correlation between the severity and/or the distribution of the dopaminergic deficit and the changes seen in cortical physiology. Such an analysis would require a larger cohort for whom both electrophysiology and imaging data are available.

Chapter 10 Conclusion

We have undertaken experiments in manifesting patients and pre-/non-manifesting gene mutation carriers with a representative autosomal dominant and recessive disease to assess the usefulness of TMS as biomarker for neurodegenerative genetic movement disorders. More precisely we aimed at assessing whether TMS may be useful 1) to give insight into underlying mechanisms of the disorders studied; 2) to detect changes early in the disease course and to monitor disease progression; and 3) to help differentiating between clinically similar diseases on the basis of certain electrophysiological patterns.

In HD, we have shown that TMS is perceptive to demonstrate changes in presymptomatic gene mutation carriers. It is known that changes in the basal ganglia and various cortical areas occur before symptom onset. This includes – as we have now shown - alterations of corticospinal output and intracortical pathways including in patients with a predicted time to onset of up to 33 years.

This time frame exceeds results from imaging findings where the process of atrophy where changes were present 9 to 11 years prior to onset.[254]

We have furthermore identified SAI as a marker of progression of the neurodegenerative process in HD. SAI is (still) normal in pre-symptomatic disease HD subjects but becomes abnormal when motor symptoms become overt. There was an association with the severity of motor signs. A regression model including SAI, CAG repeat length as the HD trait marker and age strongly predicted symptom severity. This makes SAI a possible potential biomarker in HD. Of course this needs to be tested further in larger studies.

One of the important questions with respect to biomarkers is, how well the results are able to predict endpoints on an *individual* basis. This applies to two groups of individuals. First, does the biomarker allow to subdivide those "at risk" (offspring who may or may not carry the gene) into carriers and non-carriers. This is important when children may not want to be tested or in case of other inherited disorders for which the gene has not yet been identified. Second, does the biomarker predict outcome and progression in pre-mainfesting individuals with a molecularly confirmed diagnosis? Although our attempt is only a pilot study, the data suggest that SAI may be able to distinguish between carriers and non-carriers in that a maximum SAI greater than 55 or 60 is clearly abnormal and should raise the suspicion that the subject carriers the gene. On the other hand, among the group of pre-manifesting subjects the group data suggest that there is progressive worsening of SAI over time. However, one needs to keep in mind that there is inter-individual variability and it would thus be very interesting to study the same subjects using the same parameters to look for the degree of progression of SAI abnormalities.

Similarly, in Parkin, TMS detected abnormalities not only in patients but also asymptomatic carriers. The latter are in line with functional and structural imaging studies in such cohorts. The abnormalities in patients correlated with clinical findings: CMCT was correlated to age of symptom onset. We also identified parameters - CMCT and SICI - which may help to distinguish from idiopathic disease and to select candidates for genetic testing.

Furthermore, the TMS data allow some insights into the pathophysiology of the diseases. In HD, the abnormalities of SAI suggest problems with sensory-motor integration. SAI relies on cholinergic trans-synaptic cortical pathways. As outlined above, cholinergic abnormalities have been shown in post-mortem tissue and animal models and show similarities to Alzheimer's disease.

In Parkin, our experiments add to our understanding of the pathophysiology of recessive Parkin-related parkinsonism in that compensatory mechanisms may play a part.

The review on TMS in genetic disorders has shown that most genetic conditions for which the gene has been identified have not been studied with TMS, not to mention asymptomatic carriers. As a first further step in future studies, it may be interesting to more thoroughly assess other genetic diseases and also to investigate genotype-"electro-type" correlations; thus whether specific mutations are related to certain electrophysiological changes.

An intersting role of TMS is also in normal subjects in this respect as the genetic make-up may influence not only electrophysiological response in patients, but also normals.[127] We are currently exploring the role of BDNF on TMS reponse.

Furthermore, TMS may prove useful for the identification of endophentypes.[255] Gottesman and Gould[256] defined endophenotypes as "measurable components unseen by the unaided eye along the pathway between disease and distal genotype". They represent "simpler clues to the genetics underpinning diseases than the disease syndrome itself". Such electrophysiological endophentypes may guide future genetic studies and lead to identification of genes in families which are too small for classic linkage analysis or even in (so-called) sporadic forms of disease with low penetrance.

TMS has been used to alter behaviour in both healthy controls and patients including those with genetic movement disorders (see chapter 5) In addition to using TMS as treatment per se, it may be interesting to see whether TMS response may correlate with response to treatment or even predict a good outcome (e.g. to predict surgical outcome of deep brain stimulation or response to oral medication). In this regard, we are currently studying the connection of genes-TMS-response and treatment-response in patients with Parkinson's disease.

In summary, transcranial magnetic stimulation may be a useful biomarker as tool for diagnosis and staging, as well as to predict and monitor response to intervention in (genetic) movement disorders and other neurological disorders and deserves further investigation in the future.

C) Appendix 1: Summary of electrophysiological abnormalities in genetic movement disorders (Table 5.1)

Condition	CMCT	Threshold	MEP Amplitude	CSP	SICI	ICF	SAI	RI	Blink reflex
Parkinsonismus									
Park2	Slower	Higher / normal	Increased / normal	Shorter/ normal	Normal	Normal	----	----	----
Park2 - carrier	Normal	Higher	Normal	Normal	Reduced	Normal	Reduced	----	----
Dystonia									
DYT1	----	Normal	Normal	Shorter	Reduced	Normal	----	Absent	----
DYT1 - carrier	----	Normal	Normal	Shorter	Reduced	Normal	----	Normal	----
DRD	----	Normal	Normal	Normal	Controversial (Reduced or normal)	----	----	Reduced (no change with treatment	Reduced (increased with treatment)
PxD	----	Normal	Normal	normal	Controversial (Reduced or normal)	Normal	----	Reduced	----
Myoclonus Dyst	----	Increased	----	----	Normal	----	Normal	----	----

Chorea									
HD	Normal	Controversial (normal or higher)	Controversial (reduced)	Prolonged	Reduced	Controversial (reduced or normal)	Reduced	Reduced	Abnormal (R2)
HD - carrier		Higher	----	Normal	Normal	Normal	Reduced	----	----
Ataxia									
SCA1	Slower	higher in upper limbs	----	Longer	Normal	Normal	----	----	----
SCA2	Normal	Normal; higher in lower limbs.	----	Longer	Normal	Reduced	----	----	----
SCA3	Normal	Normal	----	Normal	Normal	Reduced	----	----	----
SCA6	Normal	Normal	----	Ipsilateral SP normal; contralateral SP prolonged	Normal	Normal	Normal	----	----
Friedreich's Ataxia	Slower	Controversial	----	Normal	Normal	Normal	----	----	----

		(normal or higher)							
Myoclonus									
JME		Normal	----	Normal	Reduced	Normal	Normal	----	----
PME		Normal/ increased	----	Normal	Reduced	Normal	Paradoxical facilitation	----	----

Table 5.1 Summary of electrophysiological abnormalities in genetic movement disorders. CMCT, central motor conduction time; MEP, motor evoked potential; CSP, cortical silent period; SICI, short intracortical inhibition; ICF, intracortical facilitation; RI, reciprocal inhibition; carrier, asymptomatic / pre-symptomatic gene mutation carrier; DRD, Dopa-responsive dystonia; PxD, paroxysmal dyskinesias; Dyst, Dystonia; SCA, spinocerebellar ataxia; JME, juvenile myoclonic epilepsy; PME, progressive myoclonic epilepsy

D) Appendix 2: Publications and abstracts arising from work performed during PhD period

Main Publications in view of this thesis:

Schneider SA, Talelli P, Cheeran B, Khan N, Wood NW, Rothwell J, Bhatia KP. Corticospinal and intracortical excitability in patients and asymptomatic carriers with parkin gene mutations: a TMS study. Mov Disord (in press)

Schippling S, Schneider SA, Münchau A, Bhatia KP, Rothwell JC, Tabrizi SJ, Orth M. Short latency afferent inhibition is associated with motor symptom severity in preclinical and very early Huntington's disease (submitted)

Teo JTH, Schneider SA, Cheeran BJ, Fernandez-del-Olmo M, Giunti P, Rothwell JC. Bhatia KB. Prolonged cortical silent period but normal sensorimotor plasticity in spinocerebellar ataxia 6. Mov Disord. 2008 Feb 15;23(3):378-85.

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Schneider SA, Aggarwal A, Bhatt M, Dupont E, Tisch S, Limousin P, Lee P, Quinn N, Bhatia KP. Severe tongue protrusion dystonia: Clinical syndromes and possible treatment. Neurology. 2006 Sep 26;67(6):940-3.

Koch G, Schneider S, Franca M, Münchau A, Bäumer T, Cheeran B, Cordivari C, Rounis E, Caltagirone C, Bhatia K, Rothwell JC. The dorsal premotor-motor interhemispheric pathway is abnormal in writer's cramp dystonia. Mov Disord (epub)

Avanzino L, Martino D, Van De Warrenburg B, Schneider SA, Abbruzzese G, Defazio G, Schrag A, Bhatia KP, Rothwell JC. Cortical excitability is abnormal in patients with the "fixed dystonia" syndrome. Mov Disord (epub)

Holton JL, Schneider SA, Ganesharajah T, Gandhi S, Strand C, Shashidharan P, Barreto J, Wood N, Lees AJ, Bhatia KP, Revesz T. Neuropathology of primary adult onset dystonia. Neurology. 2008 Feb 26;70(9):695-9.

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Avanzino L, Martino D, Van De Warrenburg B, Schneider SA, Abbruzzese G, Defazio G, Schrag A, Bhatia KP, Rothwell JC. Cortical excitability is abnormal in patients with the "fixed dystonia" syndrome. Mov Disord (epub)

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Healy DG, Bressman S, Dickson J, Silveira-Moriyama L, Schneider SA, O Sullivan SS, Massey L, Shaw K, Wood NW, Lees AJ. Evidence for pre and postsynaptic nigrostriatal degeneration in the Fragile X Tremor Ataxia Syndrome. Mov Disord (in press)

Teo JTH, van de Warrenburg BPC, Schneider SA, Rothwell JC, Bhatia KP.Neurophysiological evidence for cerebellar dysfunction in primary focal dystonia. JNNP (in press)

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Draganski B, Schneider SA, Fiorio M, Kloppel S, Gambarin M, Tinazzi M, Ashburner J, Bhatia KP, Frackowiak RSJ. The basal ganglia interaction between genotype and phenotype in primary dystonia.

Schneider SA, Bhatia KP. Differential diagnosis and an approach to management of tremor.

Kägi G, Klein C, Wood NW, Schneider SA, Tadic V, Quinn NP, van de Warrenburg BP, Bhatia KP. Non-motor Symptoms in parkin-gene related parkinsonism.

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