The role of sensorimotor cortical plasticity in the pathophysiology of Parkinson's disease and dystonia

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ABSTRACT

This PhD thesis is a study of cortical electrophysiology in two basal ganglia disorders: Parkinson's disease (PD) and dystonia. Two diseases were chosen as being representative of hypokinetic and hyperkinetic movement disorders, respectively. In addition, current treatments seem to be imperfect to control many aspects of both diseases, hence the interest in exploring potential new therapeutic targets. PD and dystonia are basal ganglia diseases, but there is growing body of evidence of impaired cortical function and particularly of abnormal sensorimotor cortical plasticity in both disorders. We however still lack knowledge about functional significance of these cortical changes. Are they maladaptive or compensatory or of little functional significance?

Techniques of Transcranial Magnetic (TMS) were used to determine 1) if clinical asymmetry of early PD is reflected in hemispheric asymmetry of sensorimotor cortical plasticity and intracortical inhibition, and 2) how these electrophysiological measures change with disease progression.

We found that the hemisphere contralateral to the less affected side had preserved intracortical inhibition and a larger response to the plasticity protocol, whereas on the more affected hemisphere these were reduced. We further demonstrated that the decline in asymmetry of these measures correlated with the reduction in asymmetry of clinical symptoms, suggesting these were compensatory changes.

In dystonia patients, we investigated using TMS 1) if change of afferent input induced by botulinum toxin injections may change response to plasticity protocol in primary dystonia, and if 2) secondary and primary dystonia patients share the same pattern of electrophysiological abnormalities.

We demonstrated that sensorimotor cortical plasticity in primary dystonia is not permanent abnormality but may be transitory reduced with botulinum injections treatment. Secondary dystonia patients, as opposed to primary dystonia patients did not have enhanced sensorimotor plasticity or impaired cerebellar function. We provide evidence that different types of dystonia do not necessarily have the same neuroanatomical substrates, which might have therapeutic implications.

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THESIS OVERVIEW

Through the series of TMS experiments detailed in Chapters 3-6, this PhD thesis explores the pathophysiological aspects of sensorimotor cortical plasticity and other electrophysiologic measures in PD and dystonia.

Chapter 1 introduces the concept of brain plasticity and discuss how changes in plasticity may be relevant not only as mechanisms for compensation of neurological symptoms, but also as mechanism that cause or contribute to disease. This chapter introduces the techniques of TMS which are used to investigate PD and dystonia and then critically reviews current knowledge on electrophysiological abnormalities in both diseases.

Chapter 2 presents general methods used in the experiments described in Chapters 3-6.

Chapter 3 presents the study in which the inherent model of the clinically asymmetry of PD was used to compare electrophysiological measures between the two hemispheres. Sensorimotor cortical plasticity and intracortical inhibition were compared between the more and less affected sides in drug-naïve clinically asymmetric PD patients. It was found that the less affected hemisphere had increased cortical plasticity and preserved intracortical inhibition, while these were decreased on the more affected side.

In chapter 4 the follow-up study of the same PD patients described in Chapter 3 is presented, which aimed to define pathophysiological significance of functional cortical reorganisation in early PD. We investigated the relationship between the changes in electrophysiological measures and the progression of motor signs. Based on the results, we put forward the hypothesis that there is a compensatory role for increased sensorimotor cortical plasticity in the early stages of PD.

Chapter 5 describes set of experiments in patients with primary dystonia, intended to explore whether or not the response to TMS plasticity protocol is affected by the manipulation of the afferent input with botulinum toxin (BT) injections. The study found that the plasticity response decreased with successful BT injections and then recovered as the injections wore off. We propose that modulation of sensory afferent input by BT injections triggered subsequent reorganization of the motor cortex representation of the hand muscles, resulting in reduced sensorimotor cortical plasticity.

Chapter 6 presents the study in which we explored whether or not the primary dystonia and secondary dystonia (caused by basal ganglia lesions) share the same pattern of electrophysiological abnormalities. This study reveals that secondary dystonia patients have a normal response to experimental plasticity protocols as opposed to an enhanced response in primary dystonia patients. It also reveals differences in cerebellar functional involvement between primary and secondary dystonias.

Each of the experimental chapters 3-6 leads on to its own discussion regarding their relevance to previous work, study limitations and new insights into sensorimotor cortical plasticity which they provide.

In the final chapter, the overall conclusions are drawn from the whole work. Ideas are made about the possible clinical applications of the presented findings and also regarding directions for further work in the field.

LIST OF ABBREVIATIONS

AMT	Active Motor Threshold
APB	Abductor Pollicis Brevis
BFM	Burke-Fahn-Marsden
BG	Basal ganglia
BT	Botulinum toxin
CD	cervical dystonia
CR	conditioned response
CS	conditioning stimulus
CSP	Cortical silent period
DAT	dopamine transporter
DBS	Deep brain stimulation
FDG-PET	[18F]-fluorodeoxyglucose PET
FDI	First Dorsal Interosseous
FP	Field Potential
GABA	γ-aminobutyric acid

GPi	internal Globus Pallidus
HFS	High-frequency stimulation
ICF	Intracortical facilitation
IO curve	Input-Output curve
ISI	Interstimulus interval
LA	less affected side
LICI	Long interval intracortical inhibition
LFS	Low-frequency stimulation
LTD	Long Term Depression
LTP	Long Term Potentiation
M1	Primary motor cortex
MA	more affected side
MEP	Motor Evoked Potential
MT	Motor Threshold
NMDA	N-methyl-d-aspartate
PAS	Paired Associative Stimulation

PAS25	excitatory PAS
PD	Parkinson’s disease
SI	Stimulus intensity
SICI	Short interval intracortical inhibition
STDP	Spike-timing dependent plasticity
iTBS	intermittent Theta Burst Stimulation
TMS	Transcranial Magnetic Stimulation
rTMS	repetitive Transcranial Magnetic Stimulation
UPDRS	Unified Parkinson’s disease Rating Scale
US	unconditioining stimulus

Chapter 1 **Introduction**

1.1. Brain plasticity

Plasticity is a fundamental brain property retained throughout the lifespan, enabling the brain to modify its structure and function in response to learning and experience, aging, injury or chronic disease. It can be defined as an ability of a system to change in response to different external and internal stimuli and to remain in such a new state until the next event occurs. The concept of brain plasticity is essential to understand not only psychological brain functions such as memory, learning or acquisition of a new motor skill but also to understand the pathophysiology of common neuropsychiatric diseases (Pascual-Leone et al., 2005).

1.1.1 Adaptive plasticity vs. maladaptive plasticity in brain disorders

Brain plasticity is perhaps best described as the double edge sword with its potential beneficial and detrimental behavioural consequences.

In the context of neurological diseases, plasticity may be regarded as adaptive if it helps recovery of impaired brain function. Adaptive brain plasticity mainly comes into play after acute brain events such as stroke, traumatic or perinatal brain injury or following sensory deprivation, when functional (and structural) brain reorganisation help in improving the neurological deficit. In the stroke

literature, “brain plasticity” usually encompasses all possible mechanisms of neuronal reorganisation following ischaemic injury. From anatomical to cellular level it includes: recruitment of pathways that are functionally homologous to, but anatomically distinct from the damaged ones (for example, non-pyramidal corticospinal pathways), reinforcement of existing but functionally silent synaptic connections (at the periphery of the damaged core), dendritic arborisation, formation of new synapses and increase of synaptic strength (Rossini et al., 2003). One of the best known examples of adaptive plasticity after sensory deprivation is recovery of vision in children with acquired amblyopia caused by strabismus. Selection of visual input from one eye causes a loss of cortical synaptic connections assigned to the other eye. However, patching the opposite “healthy” eye leads to improved vision in the impaired eye if this is attempted within the period of maximal visual plasticity in the first decade of life (Johnston, 2004). The recovery in vision is possible due to reorganization of connections within previously deprived visual cortex.

Although the concept of adaptive plasticity has mainly been related to acute brain events, similar processes may have a role in chronic neurological diseases, where plasticity may be considered as adaptive if it compensates for symptoms or symptom progression. For example, in the preclinical stages of PD or Alzheimer dementia, compensatory processes may help postpone the emergence of motor signs or cognitive symptoms, respectively (Zigmond, 1997, Bezard et al., 2003). The assumption is that disease symptoms will first appear when the adaptive changes become insufficient to keep up with ongoing cell loss. Even in the symptomatic stage, when the disease continues to progress,

worsening of symptoms may be viewed as a “trade off” between the compensatory changes and the functional/structural consequences of neurodegeneration.

In contrast, brain plasticity is regarded as maladaptive when it causes or contributes to disease symptoms and/or their progression. Maladaptive plasticity has been implicated in various neurodevelopmental disorders, psychiatric diseases and adult onset neurological diseases. For example, in children with autistic spectrum disorders, several lines of evidence including findings of TMS studies, point to altered plasticity as the mechanism by which motor and cognitive behaviours are affected in these patients (Enticott and Oberman, 2013). In schizophrenia, impairment of synaptic plasticity has been shown to be associated with impaired motor skill teaching (Daskalakis et al., 2008). The opinions about the role of plasticity in Alzheimer dementia have been divided with some authors suggesting that alteration of synaptic plasticity antedate cognitive impairment and contribute to the maladaptive molecular cascade culminating in the manifestation of dementia (Pascual-Leone et al., 2011). Finally, it is believed that abnormally enhanced motor cortex plasticity contributes to the pathophysiology of dystonia in such a way that a subtle abnormality of plasticity may make some individuals susceptible to dystonia if plastic changes are pushed to their extreme by frequent repetition of particular movements (Quartarone et al., 2003).

1.1.2 Proposed mechanism of synaptic plasticity

Modulation of synaptic strength is believed to be a common mechanism of brain plasticity, shared between physiological forms of plasticity such as memory and learning and pathological forms of plasticity underlying neurological diseases.

In 1949, Donald Hebb introduced a theory of modification of synaptic strength. In his postulate on the cellular basis for learning, Hebb stated that “when an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A’s efficiency, as one of the cells firing B, is increased” (Hebb, 1949). According to Hebbian’s rule, repeated simultaneous activity in pre- and postsynaptic neuron results in an increase of synaptic efficacy, a process known as long term potentiation or LTP. Stent (1973) proposed an addition to Hebbian’s rule, considering that connections would weaken when a presynaptic neuron is active at the same time as the post synaptic neuron is inactive. The processes that decrease synaptic efficacy are referred to as long term depression or LTD. Nowadays, these two postulates are epitomised in the rule of Spike Timing Dependent Plasticity (STDP) that appears to mediate some forms of experience-dependent plasticity in vivo. In STDP, both the temporal order and the interval between pre- and postsynaptic spikes are important, so that LTP and LTD are induced when there are tight temporal correlations between the spikes of pre- and postsynaptic neurons This form of synaptic plasticity has been studied extensively in a range of models, including cultured neurons (Bi and Poo, 1998), cortical slice preparations

(Magee and Johnston, 1997) and intact animals (Jacob et al., 2007). In a typical STDP protocol, a synapse is activated by stimulating a presynaptic neuron (or presynaptic pathway) shortly before or shortly after making the postsynaptic neuron fire by injection of a short current pulse. This pairing is repeated 50-100 times at a fixed frequency. A number of studies have confirmed the importance of the temporal order of pre- and postsynaptic spiking in synaptic modification (Magee and Johnston, 1997, Bi and Poo, 1998, Caporale and Dan, 2008).

LTP can also be produced using high-frequency stimulation (HFS) of presynaptic afferents (Bliss and Lomo, 1973), whereas LTD may be produced by low- frequency stimulation (LFS) of presynaptic afferents. In brain slice preparations synaptic plasticity may be quantified as a change of field potential (FP) following experimental stimulation. For example, in a motor cortex slice, stimulating microelectrodes are placed in cortical layer II/II and FP (which is analogue to excitatory post-synaptic potential) is recorded before and after the conditioning “plasticity” protocol. The change in the FP size is a measure of synaptic plasticity.

1.1.2 Cellular mechanisms of synaptic plasticity

At excitatory glutamatergic synapses, the induction of LTP by HFS and LTD by LFS both require the activation of N-methyl-d-aspartate (NMDA) receptors and a rise in the postsynaptic Ca2+ level (Malenka and Bear, 2004). The presynaptic activation causes glutamate release while postsynaptic depolarization causes removal of the Mg2+ block on NMDA receptors, these

two processes together allowing Ca2+ influx. The amount and time course of postsynaptic Ca2+ rise depend on the induction protocol: HFS leads to fast, large Ca2+ influx, whereas LFS leads to prolonged, modest Ca2+ rise (Luscher and Malenka, 2012). In the Ca2+ hypothesis these two types of Ca2+ signals cause the activation of separate molecular pathways. Activation of Ca2+/calmodulin-dependent protein kinase II by a large Ca2+ rise is required for LTP, whereas recruitment of phosphatases such as protein phosphatase 1 and calcineurin by a modest Ca2+ increase is the basis for LTD (Luscher and Malenka, 2012).

Spike timing–dependent LTP and LTD also depend on NMDA receptor activation and the rise in postsynaptic Ca2+ level. However, LTP and LTD are not universal phenomena as the rules may differ in their details from one cell to another. Even at a single synapse, LTP produced by different patterns of stimulation may not be the same. In addition plasticity may also occur at striatal metabotropic receptors (Gubellini et al., 2004), AMPA receptors and there may also be plasticity at GABA synapses (Maffei, 2011).

1.2 Probing and measuring plasticity “in vivo”

Plasticity changes in humans are best demonstrated through behavioural changes (for example using learning and memory tasks), but may also be captured using neuroimaging and electrophysiological techniques. For instance, changes in functional activity and anatomical connectivity may be demonstrated with neuroimaging techniques, but it should be noted that imaging will reveal

presumable anatomical or functional consequences of brain plasticity, rather than directly probing the mechanisms of plasticity. More direct measures of synaptic plasticity in humans in vivo are obtained using TMS, when TMS is applied as a plastic force and the brain tendency to undergo plastic changes is then quantified.

1.2.1 Transcranial magnetic stimulation: magnetic induction as a non-invasive way to electrically stimulate the brain

The first attempts to electrically stimulate the human brain through intact scalp were made by Gualtierotti and Paterson (1954). They applied trains of stimuli over the scalp to induce motor responses in the contralateral limb. With their technique most of the current was lost by spreading through the scalp and only a small fraction reached the brain, resulting in painful and non-efficacious stimulation. Merton and Morton (1980) later introduced clinically more feasible method of transcranial electrical stimulation. They used a single high-voltage electrical pulses rather than a train of smaller pulses, which resulted in better penetration of the electrical current into the brain and relatively smaller current flow through the scalp, thus giving a more efficacious cortical stimulus. With this technique, stimulation over the motor cortex produced a twitch of contralateral body muscles and stimulation of visual cortex produced phosphenes. However, the pain was still strong enough to prevent wider clinical use. Finally, Barker et al. (1985) developed TMS, a technique for non-invasive and relatively painless stimulation of human motor cortex. Since then, TMS has been used extensively to study motor control in health and disease.

TMS relies on the principle of electromagnetic induction. A brief electrical current is passed through an insulated coil of wires placed over the scalp. This current generates a brief transient magnetic field running perpendicularly to the coil. Due to the low impedance of the scalp, skull and meninges, the magnetic field passes readily and without causing pain into the brain where it induces electric currents within the cortex. This induced electric current then activates cortical elements, resulting in an action potential or excitatory postsynaptic potential. In the motor cortex, a TMS pulse may activate corticospinal neurons directly, producing a “direct” volley of impulses in corticospinal axons (“D” ‘wave) or more commonly indirectly through transynaptic connections with cortical interneurons , producing an “indirect “volley of impulses(“I”‘ waves)(Di Lazzaro et al., 2004). The descending volley of action potentials in the corticopinal tract triggered by TMS ultimately activates target muscles. This can be seen as a muscle twitch and is detected by electromyography (EMG) as a motor evoked potential (MEP). Thus, when TMS is given over the motor cortex, the amplitude of the MEP is an indirect measure of motor cortex excitability. Similarly, the change in the MEP after applying a TMS plasticity inducing protocol is used as a measure of synaptic plasticity in the motor cortex.

When a figure-of-eight coil is held in such a direction that the TMS pulse causes electrical current to flow in a posterior-anterior direction perpendicular to the central sulcus, then corticospinal neurons are activated transynaptically and with a lower threshold. This propensity of TMS to activate corticospinal neurons transynaptically means that the response to TMS will depend on the level of excitability of cortical neurons at the time of stimulation. Indeed, it is this

tendency for transynaptic activation of corticospinal neurons that makes TMS a suitable technique for testing the excitability of intracortical synapses before and after experimental plasticity protocols.

1.2.2 TMS measures of corticospinal excitability: Motor thresholds and Input-output curve

The intensity of stimulation is expressed as a percentage of maximum stimulator output which may be adjusted in 1% gradations up to 100%. This allows for quantitative definition of corticospinal excitability in terms of the motor threshold (MT), which is defined as the lowest stimulus intensity (SI) at which MEP can be recorded in the target muscle. Thus, MT is expressed as a percentage of the maximal stimulator output and can be measured from relaxed muscle (resting motor threshold-RMT) or voluntary preactivated muscles (active motor threshold-AMT).

With increasing SI, MEP amplitude increases, allowing for the assessment of “Input-Output” (IO) curve. IO curve thus describes a relationship between intensity of stimulation and amplitude of MEPs. While MT gives information about the most excitable neurons in the representation of the target muscle, the IO curve assesses less excitable neurons, which will be activated with higher SI (neurones with a higher threshold for firing) and those more distant from the centre of the TMS coil (Hallett et al., 1999) . In healthy subjects, the shape of IO curve is approximately sigmoidal and its main features are steepness and plateau level.

1.2.3 TMS measures of intracortical inhibition and facilitation

The excitability of different intracortical interneurons within the primary motor cortex (M1) may be probed using TMS paired pulse techniques. Paired pulse experiments involve applying two stimuli, the test stimulus and the conditioning stimulus, separated in time by a varying interstimulus interval (ISI). If a suprathreshold test stimulus over M1 is preceded by a conditioning stimulus over the same cortical area, intracortical inhibition or facilitation may be tested, depending on ISI. The explanation is that the conditioning stimulus activates inhibitory or excitatory interneurons synapsing with corticospinal neurons and, depending on the ISI and on the intensity of the conditioning stimulus, the test response is either inhibited or facilitated. Several measurements of intracortical inhibition and facilitation can be probed using paired-pulses techniques, including short interval intracortical inhibition (SICI), intracortical facilitation (ICF), and long interval intracortical facilitation (LICI). TMS paired-pulse techniques thus allow the testing of the functional state of different types of intracortical interneurons. Cortical inhibitory and excitatory phenomena are subserved by different pools of interneurons: SICI is likely mediated by GABAa (Ziemann et al., 1996), ICF by NMDA (Ziemann et al., 1998c) and LICI by GABAb receptors (Werhahn et al., 1999).

Cortical silent period (CSP) is another measure of intracortical inhibition. When a TMS stimulus is delivered during voluntary contraction of a target muscle, a period of EMG silence follows the MEP (Calancie et al., 1987). Although spinal inhibitory mechanism may contribute to the early part of the cortical silent period

(up to its first 50 ms of duration), the later part is generated within the inhibitory circuits of the motor cortex. CSP is presumably mediated through GABAb receptors (Ziemann, 2004).

1.2.4 TMS as experimental plastic force

If the train of repeated TMS pulses is given over the target cortical area for a period of time (repetitive or rTMS), it is possible to induce changes in cortical excitability that outlast the period of stimulation and these are considered to reflect brain plasticity. If rTMS is used over the M1, the measure of plasticity is the change in MEP size that occurs after stimulation and that outlast the period of stimulation for minutes to hours. As a general rule, high frequency stimulation protocols (5 Hz and above) are excitatory, producing an increase in corticospinal excitability, while low frequency protocols (1-5 Hz) are inhibitory, resulting in a decrease of corticospinal excitability (Ziemann et al., 2008).

The major limitation of high frequency excitatory TMS protocols is in their potential for triggering epileptic seizures, especially if frequencies of 20 Hz and above are used (Wassermann et al., 1996). This issue is relevant, given that much higher frequencies (in the range 50-100 Hz) are used in brain slices for LTP induction. A number of other experimental TMS plasticity protocols have been invented to by-pass the problem of high frequency stimulation. The one that has been extensively used previously in both PD and dystonia and that I have used consistently through my work is paired associative stimulation (PAS).

1.2.5 Paired associative stimulation

The PAS comprise of repeated pairing of sensory afferent stimulus with TMS stimulus and relies on the principle of sensorimotor integration within the M1. Stimulation of M1 with TMS activates corticospinal neurons trans-synaptically via interneurons (Di Lazzaro et al., 2004) .The same corticospinal neurons or the interneurons within the same microcolumn projecting onto corticospinal neurons receive somatosensory input (at short latency and with high topographical specificity) via afferents from the somatosensory cortex (Rosen and Asanuma, 1972). Repeated pairing of a TMS stimulus over the cortical representation of the target muscle and an afferent stimulus evoked by electrical stimulation of the mixed peripheral nerve supplying the same target muscle may induce plastic changes, if the pairing of pulses converges on the corticospinal neurons in a precisely timed fashion (Muller-Dahlhaus et al., 2010). In the original experiment , PAS consisted of electrical stimulation of the median nerve at the wrist and a TMS stimulus over the hot spot for the APB muscle in the contralateral M1, with median nerve stimulation preceding TMS at an ISI of 25 ms (PAS25) (Stefan et al., 2000). This interval was chosen on the basis that the first component (N20) of the median nerve somatosensory-evoked potential arrives in the primary somatosensory cortex typically at around 20 ms (Allison et al., 1991) with a few extra milliseconds added on to allow for the afferent signal to be relayed from S1 to M1. Thus, the afferent signal evoked by median nerve electrical stimulation arrives in M1 synchronously, or shortly before transanssynaptic excitation of corticospinal neurons by the TMS pulse. The intensity of median nerve electrical stimulation was three times the

perceptual sensory threshold, while TMS intensity was adjusted to evoke MEPs of approximately 1 mV peak-to-peak amplitude. 90 pairs of median nerve electrical stimulation and TMS were given at a frequency of 0.05 Hz, the protocol lasting 30 minutes. Several modifications to this original protocol have been introduced, consisting of increased frequency of stimulation, with an aim to reduce the duration of stimulation. A rapid-rate paired associative stimulation introduced by Quartarone (2006a) is a variant of PAS with pairing of pulses at a rate of 5 Hz. Due to the higher frequency of stimulation, this protocol is shorten to only 2 min.

In a typical PAS experiment, the excitability of the hand motor area is first probed by single TMS pulses over the “hot spot” for a chosen muscle and 1-mV MEPs are recorded. PAS is then delivered. The measure of plasticity is the change in 1mV-MEP size when probed using the same TMS intensity as given before PAS. The PAS induced plasticity lasts for at least 30–60 min and shows a characteristic topographical specificity to the muscles innervated by the stimulated peripheral nerve (Stefan et al., 2000). The exact timing of the afferent pulses and TMS pulses is important in determining the direction of changes of cortical excitability. MEP amplitudes increase when TMS activation of corticospinal neurons follows activation of same neural elements by afferent stimulus after a few milliseconds. If however, the afferent stimulus arrives later than TMS stimulus, a reversal of the effect occurs. If an ISI of 10 ms (PAS10) is used, this results in depression of MEPs (Wolters et al., 2003). Thus, PAS is considered to be a non-invasive brain stimulation paradigm that probes STDP.

1.2.6 Between-subject and within-subject variability of TMS measures

It should be noted that even in neurologically normal subjects, there is variability in the neurophysiological and behavioural response to brain stimulation protocols (between-subject variability). As opposed to SICI which shows significant variability in the same subjects on repeated testing (within-subject variability) (Wassermann, 2002), the within-subject variability of the CSP is low, typically less than 10 % (Kukowski and Haug, 1992, Orth and Rothwell, 2004). This implies that longitudinal measurements of the CSP may be a sensitive electrophysiological marker of disease progression.

Regarding PAS, the number of non-responder among healthy subjects is considered to be between 25 and 40% (Stefan et al., 2004, Stinear and Hornby, 2005, Fratello et al., 2006, Muller-Dahlhaus et al., 2008).The cause of this variability, although not completely understood, is thought to be genetically determined (Missitzi et al., 2011) but also depends on other factors, including attention, the subjects age and hormonal levels.(Stefan et al., 2002, Inghilleri et al., 2004, Muller-Dahlhaus et al., 2008, Sale et al., 2008). However, there is much less quantitative data on the reproducibility of the PAS effect in the same subjects on repeated testing (Fratello et al., 2006, Sale et al., 2007). Although observations from these few studies have not been conclusive, the within-subject variability seems to be lower than between-subject variability. This is also suggested by the fact that the same “responders” are typically repeatedly selected for different studies as noted in several papers (Stefan et al., 2004, Sale et al., 2007).

1.2.7 Interhemispheric balance of TMS measures

It is important to note for the purpose of the studies presented here, that healthy subjects show no significant interhemispheric differences in TMS parameters of baseline corticospinal excitability such as MTs, IO curves; measures of intracortical inhibition and response to PAS protocol (Cicinelli et al., 1997, Priori et al., 1999, Ridding and Flavel, 2006). Therefore, these TMS measures may serve as sensitive markers of lateralised cortical pathology.

1.3 The contribution of TMS in revealing pathophysiology of Parkinson's disease and dystonia

PD and dystonia are BG diseases, with opposed clinical manifestation of hypokinetic and hyperkinetic movement disorder, respectively. Both diseases arise as a consequence of abnormal (disease specific) BG output, which through the thalamus reaches the motor cortex, thus affecting the motor commands for muscle activation in simple and complex movements. The BG are deep structures and as such are inaccessible for non-invasive recordings. Considerable knowledge of the pathophysiology of both diseases has been gained by recording from circuits that are under direct or indirect control of the BG, but are reachable for conventional recording techniques. In particular, TMS has gained momentum in studying cortical pathophysiology related to BG diseases. This is conceivable because the motor cortex gives a final output for all voluntary and most involuntary movements, being under the influence of convergent inputs from the BG, cerebellum and peripheral afferents.

Electrophysiological changes at cortical, brainstem and spinal cord level and within cerebellar circuits have been described in PD and dystonia. Although these have contributed to our understanding of the pathophysiology of both diseases, it is somewhat surprising that these two disorders commonly share the same pattern of electrophysiological abnormalities, even though they are at opposite ends of the spectrum of movement disorders, with PD having “too little” and dystonia “too much “movement. For example, decreased intracortical inhibition (Ridding et al., 1995a, Ridding et al., 1995b) is present in both PD and dystonia. This unexpected pathophysiological likeness between the two quite distinct diseases raises a few important issues regarding the pathophysiological significance of the abnormal electrophysiological findings in circuits under the control of the BG.

1.3.1 The significance of electrophysiological abnormalities in BG disease: Cause or consequence? Help or hindrance?

Firstly, there is an issue regarding the causal relationship between presence of electrophysiological abnormalities and clinical symptoms. This problem may be summarised in the following question: Is a certain electrophysiological finding the cause of a specific symptom or it is a consequence, or is there no relevant association between the two? For example, in dystonia, decreased intracortical inhibition (and reduced inhibition at brainstem and spinal cord level) may be responsible for some dystonic symptoms such as co-contraction of antagonistic muscles, loss of selectivity in performing independent movements and overflow of dystonia. Alternately, decreased intracortical inhibition might be a

consequence of maintaining a dystonic posture that could, through abnormal afferent input, have resulted in cortical reorganisation. Finally, neither of two interpretations may be true. Reduced inhibition is also present in parts of the body that are not affected by dystonia, and in other neurological diseases that do not feature dystonia. This suggests that changes in intracortical inhibition may represent a non-specific functional change triggered by an abnormal BG output irrespective of the underlying disease. In other words, reduced inhibition seems to be a common pattern of cortical and subcortical reorganisation that occurs when these structures are under the influence of a distorted output from the BG.

Assuming that clinical symptoms and changes in particular electrophysiological measures are related, the next important question is whether a particular electrophysiological abnormality represents a maladaptive change that contributes to the disease process, or a compensatory change that helps prevent emergence of the motor sign? Defining the pathophysiological significance of different cortical abnormalities in different types of movement disorder may be relevant for potential treatments, so as to determine in which direction to intervene, to alleviate symptoms or even to slow down disease progression. If changes are compensatory and help prevent motor symptoms emerging, then one might attempt to intervene to further enhance these changes. On the contrary, if changes are maladaptive, thus contributing to clinical symptoms and/or heralding disease progression, then it would be useful to intervene in an attempt to diminish such processes. This goal might be achieved by using non-invasive brain stimulation techniques.

With the rapidly increasing number of electrophysiological studies in PD and dystonia, it is becoming apparent that changes in the electrophysiological measures, such as corticospinal excitability and intracortical inhibition, are not specific to the disease, but rather reflect a limited repertoire of cortical "reaction" in the face of an abnormal BG output. In the hope of finding an electrophysiological marker that would be more predictive of the underlying disease, a focus of TMS research in BG diseases has switched to studies of brain plasticity.

1.3.2 Cortical abnormalities in PD as revealed by TMS and their interpretation

PD is characterised by cardinal motor symptoms of bradykinesia, rigidity and tremor. The main pathological substrate is dopamine cells death in the substantia nigra pars compacta with consequent striatal denervation. Although the main pathological burden is within the nigrostriatal system, functional changes arise also in the structures downstream from the striatum, including the motor cortex, as a consequence of an abnormal BG output (Obeso et al., 2008). A second source of cortical dysfunction is reduced dopaminergic projections from the midbrain directly to the motor cortex (Gaspar et al., 1991). Treatments with L-DOPA or dopamine receptor agonists successfully relieve the motor symptoms of PD, but this can become complicated by motor fluctuations and dyskinesias during the course of disease. Deep brain stimulation (DBS) of the subthalamic nucleus or the internal globus pallidus (GPi) improve motor symptoms even in patients with advanced disease including motor

complications, but the surgical approach is limited by many contraindications to surgery and the risk of surgical complications. Studying the motor cortex involvement in PD and its relationship to the motor symptoms may reveal functional reorganisation that occurs in the face of dopamine loss and thus may have treatment implications, given the accessibility of the motor cortex to non-invasive brain stimulation techniques. Defining the mechanisms of sensorimotor cortex reorganisation may thus be helpful for building the most appropriate non-invasive brain stimulation protocols with view to treating motor symptoms of PD.

In trying to understand how the abnormal BG output in PD causes the cardinal motor symptoms, the motor cortex has been increasingly investigated. Indeed, different aspects of motor cortex function as revealed by TMS are found to be abnormal in PD and are discussed below:

Motor thresholds and IO curve in PD

While there is no difference between PD patients and healthy controls in simple measures of corticospinal excitability, such as RMT or AMT (Ueki et al., 2006), IO curves are steeper in patients comparing to controls (Valls-Sole et al., 1994, Chen et al., 2001). This is thought to be an expression of increased corticospinal excitability in PD. However, a difficulty with this interpretation is that background muscle activity itself influences the size of MEP for a given stimulus intensity. Therefore, it may be that the steeper IO curve represents a confounding effect of rigidity rather than increased cortical excitability.

Intracortical inhibition in PD

Several measured of intracortical inhibition are also impaired in PD. For example, shortening of CSP comparing to controls has been repeatedly reported (Priori et al., 1994, Nakashima et al., 1995, Berardelli et al., 1996, Manfredi et al., 1998). CSP is shorten in the hemisphere contralateral to the clinically more affected side compared to the less affected side in early asymmetric PD patients (Cantello et al., 2007) and dopaminergic medications prolong/ normalize the CSP, providing the evidence that the deficit in the CSP is dopamine related.

SICI is also reduced in PD patients when they are tested “off” dopaminergic medications (Ridding et al., 1995a), with this abnormality being present from the early stages of the disease (Buhmann et al., 2004). There is still ongoing debate about the exact cortical origin of the reduced SICI in PD. Some authors suggest that a decreased SICI reflects decreased threshold of excitatory neurons mediating intracortical facilitation (MacKinnon et al., 2005, Ni et al., 2013), while the classic view is that SICI reflect dysfunction of inhibitory GABAa interneurons.

The interpretation of the functional significance of reduced intracortical inhibition in PD is not straightforward. A classical view is that deficient inhibition is an indirect expression of the dopaminergic deficit which, by distorting BG output, affects the motor cortex (Ridding et al., 1995a). An alternative hypothesis is that reduced cortical inhibition serves as a compensation for bradykinesia (Cunic et

al., 2002). For example, in healthy subjects SICI is reduced during voluntary muscle contraction (Ridding et al., 1995c), and even prior to the onset of movement (Reynolds and Ashby, 1999). Accordingly, in PD reduced SICI at rest may serve to facilitate the initiation of movement, representing an adaptive motor strategy that compensates for slowness of movement

.

Plasticity in Parkinson's disease

Dopaminergic deficit is clearly related to abnormal plasticity mechanisms at corticostriatal synapses in animal models of PD. In the 6-hydroxydopamine parkinsonian rat, complete dopaminergic denervation decreases both LTP and LTD, while incomplete dopaminergic selectively affects LTP in corticostriatal synapses. While it has not been possible to test for plasticity changes in corticostriatal synapses in vivo in PD patients, plasticity of the sensorimotor cortex has been extensively investigated. This was found to be abnormally reduced and even absent in advanced PD patients when tested "off" dopaminergic treatment (Morgante et al., 2006, Ueki et al., 2006, Suppa et al., 2011, Kacar et al., 2012, Kishore et al. 2012) , suggesting widespread functional abnormalities that are not confined to the BG . Dopamine plays a key role in the modulation of mechanisms of synaptic plasticity, therefore it is not surprising that the response to an experimental plasticity protocol recovers with dopaminergic treatment (Morgante et al., 2006, Ueki et al., 2006). Reduced motor cortex plasticity may be the consequence of dopamine loss within nigrostriatal system, that via abnormal BG output affect the cortex, or it may be a result of the dopaminergic deficit within M1. However, it should be noted that

not all studies reported abnormally reduced plasticity in PD. For example, Bagnato et al. (2006) reported increased plasticity in the sensorimotor cortex in PD patients, namely a stronger PAS-induced increase of the MEP amplitude with a spread of the PAS effect in to non-target muscles compared to a healthy control group. In this study, PAS response normalised when patients were retested “on” medications. These apparently contradicting findings suggest that the motor cortex may undergo various stages of functional reorganisation, rather than being in a fixed disease- predetermined state.

Maladaptive plasticity at corticostriatal synapses has been implicated also in the genesis of Levodopa induced dyskinesias. Depotentiation is a form of synaptic plasticity that implies a reversal of established LTP by a low-frequency stimulation protocol and also depends on dopaminergic signalling. Depotentiation is absent in corticostriatal synapses in an experimental model of l-DOPA-induced dyskinesias (Picconi et al., 2003). Similarly, dyksinetic but not non-dyskinetic PD patients have impaired depotentiation in the M1, again probably reflecting abnormal mechanisms of synaptic plasticity that generalises across the whole BG-thalamo-cortical circuit (Huang et al., 2011).

In summary, PD is traditionally understood as a disorder with reduced motor cortex plasticity. Even though considerable experimental evidence suggests that this is the case in advanced PD, preclinical stages and patients in early stages of disease have been much less investigated. If symptoms of neurodegenerative diseases are regarded as a trade-off between compensatory mechanisms and irreversible cell loss, it is possible that changes in plasticity in

PD reflect a dynamic process, that may initially have a compensatory role, while later becoming ineffective or even maladaptive.

1.3.3 Cortical abnormalities in dystonia as revealed by TMS and their interpretation

Dystonia is a hyperkinetic movement disorder, featuring repetitive twisting movements and sustained abnormal postures of affected parts of the body. Dystonia is classically thought to be a basal ganglia disorder and there are several lines of evidence to support this: (i) Lesions of the BG and its connections may cause dystonia. (ii) Abnormal activity or subtle changes in BG structure have been demonstrated in primary dystonia using different imaging techniques. (iii) Dystonia often occurs in other BG diseases or may be the main symptom of nigrostriatal dysfunction, i.e. acute dystonic reaction and tardive dystonia. (iv) Finally, dystonic symptoms may be cured or significantly improved with DBS.

However, there are a limited number of experimental studies including electrophysiological recordings from BG structures, the reason being the relative rarity of the disease and the invasive nature of such studies. Therefore, only patients undergoing deep brain stimulation surgery have been studied (Vitek et al., 1999, Zhuang et al., 2004, Starr et al., 2005, Tang et al., 2007). Another source of information on abnormal motor control in dystonia was gathered from electrophysiological studies that involved the cortex, brainstem, spinal cord and recently, cerebellum.

Loss of inhibition in dystonia

A consistent finding across neurophysiological studies in dystonia has been a loss of inhibition at different CNS levels, including spinal cord, brainstem and motor cortex (Berardelli et al., 1985, Nakashima et al., 1989, Ridding et al., 1995b). It seems logical to relate the loss of inhibition to some dystonic manifestations such as non-selective and prolonged muscle contraction. However, deficient inhibition cannot be the sufficient explanation for the genesis of dystonic symptoms, because the same abnormality is also present in parts of the body not affected by dystonia (Sommer et al., 2002), in clinically unaffected carriers of DYT1 mutations (Edwards et al., 2003), in other hyperkinetic movement disorders such as tics (Ziemann et al., 1997) and even in hypokinetic movement disorders such as PD (Ridding et al., 1995a).

Plasticity in dystonia

Maladaptive sensorimotor cortical plasticity has been put forward as one of the major pathophysiological features of dystonia (Quartarone and Pisani, 2011). It has been showed that patients with primary dystonia have enhanced response to different experimental plasticity protocols (Quartarone et al., 2003, Edwards et al., 2006, Weise et al., 2006, Tamura et al., 2009). In the initial study on PAS response in writer's cramp, patients had two main abnormalities. Firstly, the PAS response was enhanced in the target APB muscle comparing to controls and secondly, there was a loss of topographic specificity in patients, with an increase of MEP in the non-target FDI muscle after PAS that was not present in

healthy participants (Quartarone et al., 2003). Yet, not all studies reported an increased response to PAS in primary dystonia patients (Meunier et al., 2012). Another way to look at plasticity changes is by indirect mapping of sensory and motor cortices, using TMS or functional imaging. In patients with primary dystonias, mapping studies showed enlargement of sensory receptive fields and blurring of margins of motor maps with overlap between representations of adjacent muscles (Thickbroom et al., 2003).

An important feature that may link the role of maladaptive plasticity to development of dystonic symptoms is that focal limb dystonia is typically triggered by a period of intensive training of a particular movement. In a monkey model of dystonia, overtraining in specific hand movements may also trigger the appearance of symptoms that resemble human dystonia (Byl et al., 1996). The somatosensory cortex of these animals undergoes functional reorganisation, resulting in enlargement and overlapping of receptive fields of individual digits. The idea is that overtraining itself triggers functional changes in sensory and motor cortices, leading to abnormal sensorimotor integration that somehow results in dystonic symptoms. However, an important question is why only some human subjects develop dystonia after excessive training whereas others do not. The answer might be in the two- hit hypothesis, which suggests that dystonia develops when use-dependent environmental factors coexist with inherently abnormal mechanisms of plasticity within the sensorimotor cortex. Thus, abnormal sensorimotor cortex plasticity may have a role in the pathophysiology of dystonia, so that individuals with an excessive tendency to form an association between sensory input and motor output may develop

symptoms if plastic changes are pushed to an extreme by frequent repetition of movement (Quartarone and Pisani, 2011).

However, it should be noted that the two-hit explanation account only for task-specific primary dystonias, as it doesn't consider other forms dystonia in which there is no obvious role for over-training of the affected body parts. These include other focal and generalised primary dystonias or secondary dystonias. It might be possible that in these other forms of dystonia abnormalities of the plasticity mechanisms coexist with other factors that may trigger dystonia, for example BG lesions in the case of stroke or perinatal injury, neuroleptic drug use in the case of drug induced dystonia or still unknown environmental factors in the case of primary dystonias that are not task specific. Alternatively, enhanced mechanism of synaptic plasticity might be a trait specific for primary dystonia only.

Finally, distorted plasticity is also present in cortical representations of the body parts that are not affected by dystonia (Quartarone et al., 2008) and in professional musicians who do not develop occupational dystonias (Rosenkranz et al., 2007), further complicating the issue of pathophysiological significance of abnormal plasticity in primary dystonias.

In summary, it has been traditionally understood that enhanced motor cortex plasticity represented a maladaptive trait for the development of dystonic symptoms. While this might be the case in primary (including genetic forms) dystonias, increased plasticity might not be a feature of all dystonias. For

example, patients with psychogenic dystonia have normal response to experimental plasticity protocols (Quartarone et al., 2009), while other forms of dystonia including secondary forms have not been hitherto investigated. Thus, there may be no uniform hypothesis to explain the pathophysiology of all dystonias, but rather, different forms of dystonia may have diverse pathophysiological backgrounds.

Neuroanatomical network of dystonia

Several studies have addressed the link between BG output and cortical, brainstem and spinal cord functional abnormalities, by investigating the changes in electrophysiological measures within these circuits following DBS of GPi in patients with primary dystonia.

When abnormal BG output is modulated by DBS treatment, this results in normalisation of previously reduced inhibition at different CNS levels (Tisch et al., 2006a, Tisch et al., 2006b) and importantly the normalisation of sensorimotor cortical plasticity (Tisch et al., 2007, Ruge et al., 2011).

In healthy subjects, the cerebellum modulates the extent and duration of the response to sensorimotor plasticity protocols, in such a way that cerebellar cortex excitation prevents the PAS inducing a sensorimotor plasticity in the primary motor cortex (Hamada et al., 2012b, Popa et al., 2012), whereas there is some evidence that cerebellar cortex inhibition makes the PAS more efficient at inducing plasticity (Popa et al., 2013). In patients with writer's cramp,

cerebellar cortex excitation and inhibition are both ineffective in modulating sensorimotor plasticity, suggesting that altered cerebellar processing of incoming afferent information may result in maladjusted sensorimotor integration and consequently abnormal response to PAS in primary dystonia (Hubsch et al., 2013).

There may also be a link between distorted afferent input and abnormal cortical plasticity in dystonia. For example, in focal limb dystonia altered hand cortical maps which are index of maladaptive cortical plasticity are normalised if afferent input is modified by botulinum toxin injections into dystonic muscles (Thickbroom et al., 2003).

Dystonia may be thus be regarded as an abnormality of broad functional motor network, which apart from BG include sensorimotor cortex, sensory afferent input, the cerebellum, brainstem and spinal cord. The assumption is that abnormalities in sensorimotor cortical plasticity in dystonia should not be regarded as an independent fixed state, but rather as a dynamic functional reorganisation of motor cortex influenced by inputs from different nodes in the broad dystonia network. Furthermore, different types of dystonia might not necessary share the involvement of the same nodes in the dystonia network, but may rather have different neuroanatomical substrates.

1.4. Aim of the PhD study

The aim of my PhD research was to gain further knowledge on significance of sensorimotor cortical plasticity changes (and other cortical electrophysiology measures) in PD and dystonia. I tried to achieve this by studying relationship between changes in these measures and changes in clinical manifestation of disease in PD and primary dystonia. In addition, I compared sensorimotor cortical plasticity and other electrophysiological measures between primary and secondary dystonia patients to see if different types of dystonia share the same neurophysiological pattern.

Chapter 2 **General Methods**

In all the work within this thesis, I have used TMS techniques to study corticospinal excitability, intracortical inhibition and sensorimotor cortical plasticity in PD patients, different group of dystonia patients and healthy participants.

2.1. Participants

PD patients and patients with primary and secondary dystonia were recruited from the Movement Disorder Outpatient Clinics at the National Hospital for Neurology and Neurosurgery in London and occasionally from the collaborating institution depending on the study (Sapienza University at Rome and University of Seville). Healthy participants were recruited from the list of healthy participants maintained by Movement Disorders Group of the Sobell Department of Motor Neuroscience and Movement Disorders. The exclusion criteria for all participants were in accordance to guidelines for use of repetitive TMS (rTMS) in research and included history of epileptic seizures and implanted metal device (Wassermann et al., 1996, Rossi et al., 2009). Demographic and clinical characteristics of the patients are given in experimental chapters (Chapters 3-6).

Written informed consent was obtained from all participants. All studies were approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki.

2.2. Electromyographic recordings

Participants were seated comfortably in an armchair and were instructed to relax arm and hand muscles. EMG recordings were made from hand muscles: abductor pollicis brevis (APB) and first dorsal interossei (FDI) or abductor digiti minimi (ADM) muscles on the side contralateral to stimulated cortex. Ag-AgCl surface electrodes with a belly-tendon montage were used, with a ground electrode placed over the wrist. The level of background EMG activity was monitored and trials with background EMG activity were rejected online. The background EMG area for at least the 200 ms preceding the TMS pulse was measured in all recorded trials and the EMG root mean square amplitude calculated to ensure comparability of the baseline activity across different experiments in patients (within-subjects) and between patients and healthy participants (between-subjects). EMG signals were amplified (1000x) and band-pass filtered (bandwidth 20Hz to 2 kHz) with a Digitimer D360 amplifier (Digitimer, UK), acquired at a sampling rate of 5 kHz through a 1401 laboratory interface (Cambridge Electronic Design, Cambridge, UK) and stored on a PC. The EMG traces were analysed “off- line”, using customized Signal® software version 4.00.

2.3. Transcranial Magnetic Stimulation

Single TMS pulses of the M1 were applied using Magstim 200^2 magnetic stimulator with a monophasic current waveform (Magstim Company, Carmarthenshire, Wales, UK). For paired pulses techniques, two Magstim 200^2 stimulators were coupled via a Bistim module. Stimulators were connected to a standard figure-of-eight TMS coil (diameter 70 mm). The intersection of the coil was held over the "hotspot", tangentially to the skull with the handle pointing backwards and laterally at an angle of ~45 degrees to the sagittal plane in order to generate a posterior–anterior current in the brain (Kaneko et al., 1996). The "hot spot" was marked on the participant's head over the optimal scalp positions for eliciting MEPs of maximal amplitudes in the contralateral APB muscle. The same hot spot was used for assessing the MEPs in other muscles (Stefan et al., 2000).

2.3.1. Corticospinal excitability

Active and resting motor threshold were determined according to the standard definitions (Rossini et al., 1994). RMT was defined as the minimum intensity that evoked a peak-to-peak MEP of 50 μV in at least 5 out of 10 consecutive trials in the relaxed APB muscle. AMT was defined as the minimum intensity that elicited a reproducible MEP of at least 200 μV in the tonically contracting APB muscle in at least 5 out of 10 consecutive trials, while subject was performing contraction of ABP at approximately 10–15% of maximum voluntary contraction.

Single 1mV-MEPs were recorded using a SI adjusted to produce MEP of approximately 1 mV amplitude in the relaxed APB muscle and this intensity was kept constant for assessment of changes of 1mV-MEPs after PAS. IO curves were assessed by recording four MEPs at raising SI, increasing in 10% steps, as indicated in details in each experimental chapter.

2.3.2. Intracortical inhibition

SICI and ICF were assessed with paired-pulse paradigm (Kujirai et al., 1993). The intensity of the test stimulus was 1mV- MEPs intensity. In studies on PD patients described in chapters 3 and 4, the intensity of the conditioning stimulus (CS) was 90% of RMT, an intensity known to produce a net loss of inhibition in PD (MacKinnon et al., 2005). In studies on dystonia patients (chapters 5 and 6), the intensity of CS was set at standard 80% of AMT. SICI and ICF were assessed at rest, using ISIs as indicated in each experimental chapter. For SICI and ICF 10 MEPs were collected for each ISI and for the test stimulus alone. For assessment of CSP, 20 single TMS pulses were applied at an intensity of 120% RMT, while patients performed a constant contraction of APB at 20% of maximum voluntary contraction.

2.4. Paired Associative Stimulation

For studies described in chapters 3, 4 and 6, I used excitatory PAS protocol (PAS25), which consisted of 200 electrical stimuli to the median nerve at the wrist paired with TMS stimuli over the APB hot spot, given at the rate 0.25 Hz

(Ziemann et al., 2004). Each TMS stimulus was preceded by an electrical stimulus by 25 ms (ISI 25ms). Intensity of electrical stimulus was 300% of the perceptual threshold; while TMS intensity was adjusted to the intensity that produced 1mV-MEP in relaxed APB muscle. Median nerve electrical stimulation was applied through a bipolar electrode, with the cathode positioned proximally (Digitimer DS 7 stimulator; Digitimer Ltd, Welwyn Garden City, Herts, UK). The electrical pulses were constant current square wave pulses with a pulse width of 200 μs.Subjects were instructed to look at their stimulated hand and to report every 20th peripheral electrical stimuli they perceived in order to ensure comparable attention levels between sessions. Number of errors in counting peripheral nerve stimuli was noted. In the study described in chapter 5, a rapid PAS (rPAS) protocol was used, with details explained in that chapter. In all experiments, to assess the effect of PAS (or rPAS) on 1mV-MEPs, 20 MEPs were recorded before PAS and at each time point after PAS.

2.5 Statistical analysis

Distribution of data was assessed using Shapiro–Wilk test of normality. Greenhouse-Geisser method was used where necessary to correct for non-sphericity. When data were normally distributed, parametric tests (ANOVA) with post-hoc Tukey test were used. For non-normally distributed data and for ordinal data, non-parametric tests were used as described in the experimental chapters. The significance was pre-set at $p \leq 0.05$. Unless otherwise stated, data are given as mean +/- standard error of the mean (SEM).

Chapter 3 **Interhemispheric asymmetry in sensorimotor cortical plasticity and intracortical inhibition in early Parkinson's disease**

In the following set of experiments we studied if clinical asymmetry of the motor symptoms in early, drug- naïve PD patients is reflected in an asymmetry of cortical TMS measures. In the "model" of early asymmetric PD, the more affected side is in the more advanced stage of the disease, while less affected side is still asymptomatic or very mildly symptomatic, despite considerable dopaminergic loss even in the striatum corresponding to the less affected side. The hypothesis was that if there is any functional change that prevents motor symptom progression it is likely to be detected in the hemisphere contralateral to the less affected side. Thus, we compared the corticospinal excitability, intracortical excitability and sensorimotor cortical plasticity between the hemispheres contralateral to the clinically less and more affected side.

The work presented in this Chapter was originally published in the form of a research article: Kojovic M, Bologna M, Kassavetis P, Murase N, Palomar FJ, Berardelli A, Rothwell JC, Edwards MJ, Bhatia KP. Functional reorganization of sensorimotor cortex in early Parkinson disease. *Neurology*. 2012 May 1;78(18):1441-8.

3.1 Summary

Several neurophysiological measures known to be abnormal in the M1 of patients with advanced PD were tested in the both hemispheres (contralateral to the more and less affected side) of 16 newly diagnosed and drug naive PD patients and compared with 16 age-matched healthy participants. LTP-like effects were probed using a PAS protocol. We also measured SICI, ICF, CSP and IO curves. We found that the hemisphere contralateral to the less affected side had preserved intracortical inhibition and a larger response to the plasticity protocol than healthy participants. In the hemisphere contralateral to the more affected side, there was no response to the plasticity protocol and inhibition was reduced. There was no difference in the IO curves between sides or between PD patients and healthy participants. Increased sensorimotor cortical plasticity in the hemisphere contralateral to the less affected side is consistent with a functional reorganisation of the sensorimotor cortex and may represent a compensatory change that contributes to delaying the onset of clinical signs. Alternatively, it may reflect a maladaptive plasticity that provokes onset of the symptoms. As seen in the hemisphere contralateral to the more affected side, plasticity deteriorates as the symptoms progress. The rate of change in the interhemispheric difference of the PAS response over time could be developed into a surrogate marker of disease progression.

3.2. Introduction

Motor signs in PD appear when striatal dopamine is depleted beyond the critical threshold of approximately 60-80 % (Lee et al., 2000). Neuropathological and neuroimaging evidence suggests that changes in the nigrostriatal system compensate for dopamine deficiency (Zigmond et al., 1990, Kaasinen et al., 2000, Lee et al., 2000, McCallum et al., 2006, Appel-Cresswell et al., 2010, de la Fuente-Fernandez et al., 2011). However, given the extent of preclinical dopaminergic denervation (Tissingh et al., 1998), it is conceivable that compensatory changes extend beyond the nigrostriatal circuit.

Clinically asymmetric PD patients represent a valuable model for studying compensatory reorganisation within the motor system since functional changes that prevent symptom progression may possibly be present in the hemisphere contralateral to the less affected side. A previous [18F]-fluorodeoxyglucose PET (FDG-PET) study provided little evidence that this might be the case (Tang et al., 2010). Asymmetric patients had an equally abnormal metabolic pattern in the cortex and subcortex of both hemispheres (except within the putamen) (Tang et al., 2010). However, an apparent absence of metabolic asymmetry in the sensorimotor cortex, a major output of basal ganglia-cortical loops, could reflect insufficient sensitivity of metabolic measures.

In this study we measured the excitability of circuits in the sensorimotor cortex of clinically asymmetric drug naive PD patients with TMS techniques known to be sensitive to dopaminergic deficit. These involved PAS, a method that assesses long term potentiation LTP- like plasticity at cortical synapses (Stefan et al., 2000) and which relies on sensorimotor integration of afferent input and

motor output, that are known to be impaired in PD (Lewis and Byblow, 2002). In addition, we employed measures of intracortical inhibitory and excitatory function. We compared these measures between the less and more affected hemispheres in the patients and we contrasted them with those of healthy participants.

3.3 Methods

3.3.1 Participants

Sixteen newly diagnosed, drug naive patients with clinically asymmetric idiopathic PD (11men, 5 women, mean age 59 years, range 34-73 years) (**Table 3.1**) and sixteen age -matched healthy participants (11men, 5 women, mean age 60 years, range 35-73 years) were included in the study. Idiopathic PD was diagnosed according to the UK Parkinson's Disease Society Brain Bank criteria (Hughes et al., 1992) and further confirmed by abnormal dopamine transporter (DAT) SPECT in all patients. Clinical disease severity was assessed with the motor section (items 3.1–3.18) of the MDS-UPDRS scale (Goetz et al., 2008a). For the less and more affected side, the motor subscore was calculated from the sum of items 3.3 to 3.8 and 3.15 to 3.17 for each side (lateralised UPDRS score). None of the participants was on any medications that are known to affect the measurements performed. All participants were right-handed.

Table 3.1 Clinical and demographic characteristics of Parkinson's disease patients

Patient	Age (years)	Gender	Less affected side	Disease duration (months)	UPDRS less affected side	UPDRS more affected side	Motor UPDRS total
1	34	F	R	28	1	20	24
2	71	M	L	28	1	9	18
3	44	M	R	24	0	8	12
4	66	M	R	9	1	6	9
5	62	F	L	10	0	12	12
6	67	M	L	28	1	4	9
7	64	M	L	24	2	13	26
8	50	M	L	48	7	18	32
9	63	M	L	36	2	4	6
10	58	M	L	5	2	3	6
11	62	M	R	6	0	9	9
12	73	F	L	60	1	13	19
13	61	F	R	3	2	4	7
14	52	F	R	8	1	6	8
15	65	M	L	10	1	6	12
16	48	M	L	24	3	23	33
Mean ± SEM	**58.7 ± 2.6**	**11M:5F**	**10L:6R**	**22 ± 4.1**	**1.6 ± 0.4**	**9.9 ± 1.5**	**15.1 ± 2.3**

Abbreviations: UPDRS, Unified Parkinson’s Disease Rating Scale; M,male; F, female ; R, right;L, left

3.2 EMG recordings and TMS

Details are given in methodological chapter 2. EMG recordings were made from APB and ADM muscles. IO curves were assessed by recording four MEPs at each of ten stimulation intensities, increasing in 10% steps from 80% to 170% of RMT.SICI was assessed using ISIs of 2, 3 and 4 ms and ICF at ISI of 15 ms.

3.3.3 Experimental design

Patients were tested on both hemispheres, corresponding to the more and less affected side in two different TMS sessions, separated by a week. The order of the tested hemisphere (affected vs. unaffected) was randomised between subjects. Healthy participants were tested on the dominant hemisphere only, since there is no evidence of a difference in sensorimotor cortical plasticity and other TMS measures between the dominant and non-dominant hemisphere. In each session we measured RMT, AMT, 1mV MEP, IO curve, SICI, ICF and CSP. We then delivered PAS and assessed the effect of this conditioning protocol on corticospinal excitability (RMT, AMT and 1mV- MEPs) and CSP at three time points: 0, 15 min and 30 min after PAS (**Figure 3.1**).

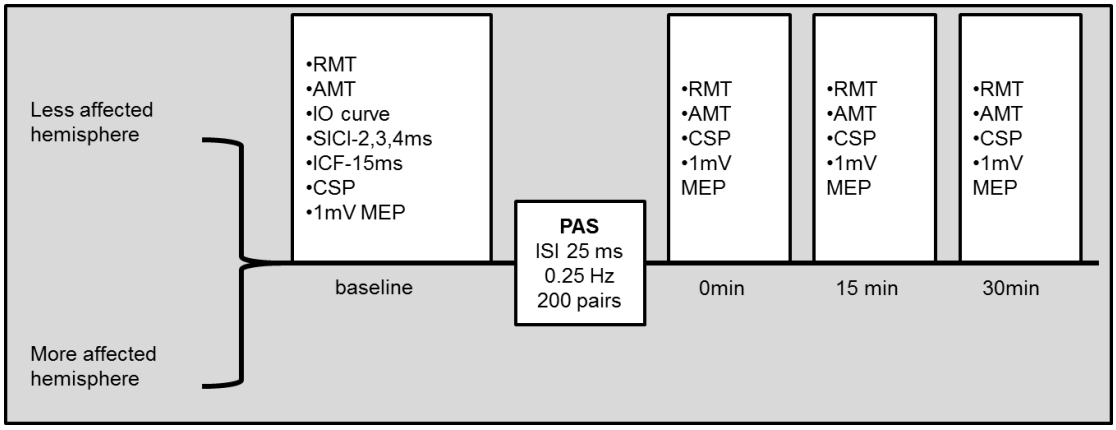


Figure 3.1 Experimental design

3.3.4 Statistical analysis

We used Wilcoxon's signed-rank test to compare differences in the UPDRS scores between less and more affected side and to compare age between PD patients and healthy participants. Chi-square test was used to compare gender distribution between PD patients and healthy participants. The TMS parameters between the hemispheres of PD patients and healthy participants were compared using 2-way ANOVAs with a factor GROUP (less affected vs. more affected side vs. healthy participants) as a between-subject factor. For IO curves the factor STIMULUS INTENSITY (10 levels of stimulator output intensity) was used as within-subjects factor. For SICI, ISI (3 levels: normalised MEP size at 2, 3 and 4 ms) was used as a within-subject factor. PAS produced by stimulation of median nerve has different effects on MEPs evoked in median and non-median innervated muscles (Stefan et al., 2000). Thus the effects on MEPs in the APB and ADM muscles were evaluated in separate 2-way ANOVAs for each muscle with TIME (4 levels: before PAS and 0, 15 and 30 min after PAS) as a within-subject factor. The effect of PAS on CSP was evaluated using TIME (3 levels: normalised CSP duration at 0, 15 and 30 min after PAS) as a within-subject factor. Conditional on a significant F-value, to explore the strength of main effects and patterns of significant interactions we used post hoc Tukey HSD test and follow-up ANOVAs, respectively. Possible correlations

between clinical and demographic data and TMS measures were evaluated with Spearman correlation analysis.

3.4. Results

3.4.1 Clinical and demographical data

No differences in age and gender distributions were found between PD patients and healthy participants. As expected, there was a significant difference in lateralised UPDRS scores between the less and more affected side in PD patients (**Table 3.1**), due to higher scores on the more affected side (paired sample t-test, $p< 10^{-3}$).

3.4.2 Corticospinal excitability and EMG root mean square amplitude

At baseline, there was no difference in RMT, AMT, 1mV MEPs or resting EMG root mean square between the hemispheres in PD patients or between patients and healthy participants. For IO curves ANOVA showed an expected effect of STIMULUS INTENSITY ($F (9, 423) =73.4$; $p <10^{-3}$). Factor GROUP and the interaction GROUP X STIMULUS INTENSITY were non-significant, indicating no difference in baseline corticospinal excitability between the groups.

3.4.3 SICI

ANOVA revealed a difference in SICI between groups (factor GROUP (2, 45) =6.28; p=0.004), due to overall reduced SICI in the hemisphere contralateral to the more affected side compared with the hemisphere contralateral to the less affected side (p=0.01) and with healthy participants (p=0.007) (**Figure 3.2**). There was no difference in SICI between the hemisphere contralateral to the less affected side and healthy participants. Factor ISI was also significant (F (2, 90) =14.8; p $<10^{-3}$), due to less SICI at 4 ms compared to 2 and 3 ms (p $=10^{-3}$ and p$<10^{-3}$, respectively) across all 3 groups. (GROUP X ISI interaction was not significant). The correlation analysis between lateralised UPDRS score and the averaged amount of SICI at 2, 3 and 4 ms (expressed as a ratio to unconditioned MEP) revealed that more severe symptoms were associated with greater reduction in SICI (R= 0.42; p=0.017) (**Figure 3.3**).

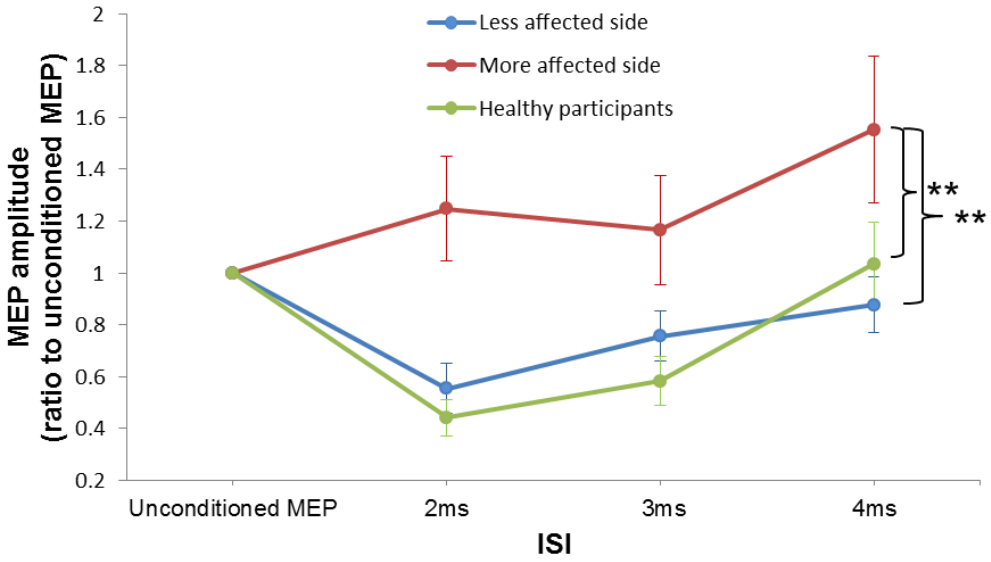


Figure 3.2 Short interval intracortical inhibition

In PD patients SICI is preserved in the hemisphere contralateral to the less affected side and does not differ from SICI in healthy participants. In the hemisphere contralateral to the more affected side SICI is reduced. Data is plotted as a ratio to the unconditioned MEP amplitude. .

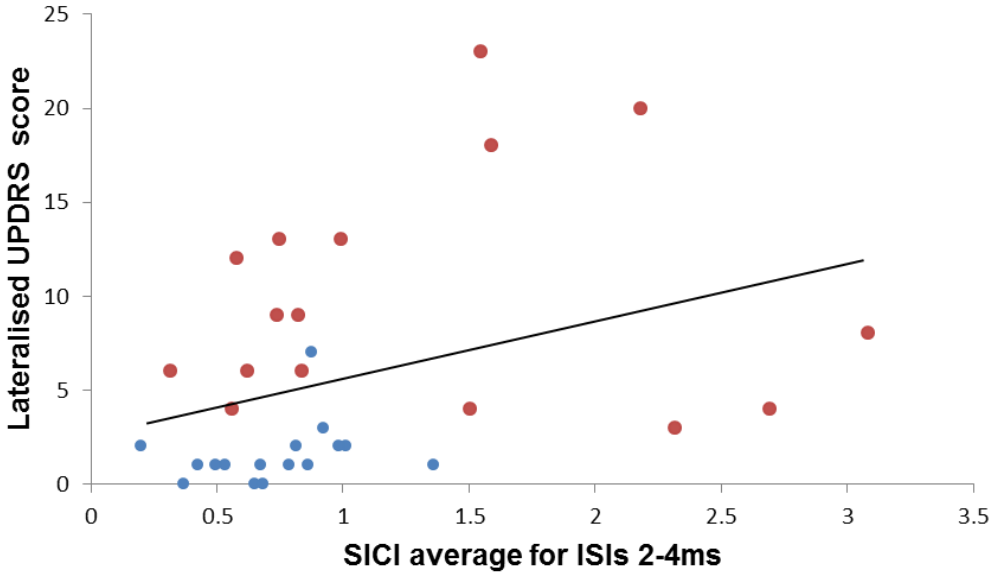


Figure 3.3 Correlation analysis between SICI and clinical severity of PD

Averaged SICI (for ISI 2, 3 and 4ms, expressed as a ratio to the unconditioned MEP) positively correlates with lateralised UPDRS scores. A higher SICI ratio corresponds to less SICI and therefore a positive correlation indicates that the greater reduction in SICI is associated with more severe motor symptoms. Blue markers and red markers correspond to the values from the hemisphere contralateral to the less and more affected side, respectively. If values from less and more affected sides are plotted separately, there is however no significant correlation. (** $p \leq 0.01$)

3.4.4 ICF

For ICF, ANOVA revealed no group difference (F (2, 37) =0.56; p =0.94) (**Figure 3.4**).

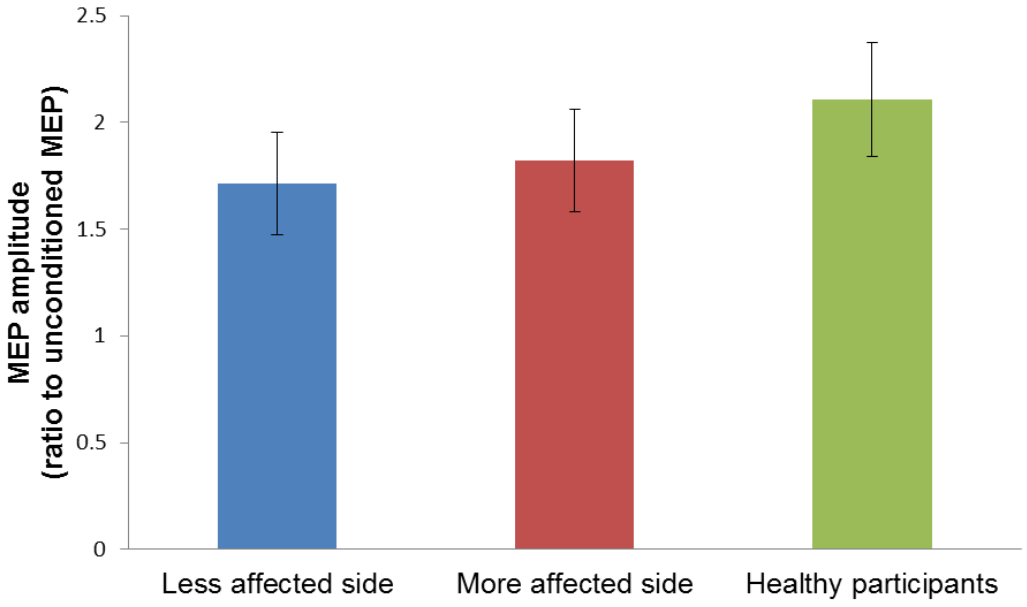


Figure 3.4 Intracortical facilitation at ISI 15 ms

There is no difference in ICF between the hemispheres in PD patients or between patients and healthy participants. Data is plotted as a ratio to the unconditioned MEP amplitude.

3.4.5 CSP

At baseline, ANOVA revealed differences in the CSP duration between groups. ($F(2, 45) = 5.73$; $p = 0.006$), due to a shorter CSP in the hemisphere contralateral to the more affected side compared with the less affected side ($p = 0.02$) and healthy participants ($p = 10^{-3}$). There was no difference in baseline CSP between the hemisphere contralateral to the less affected side and healthy participants (**Figure 3.5**).

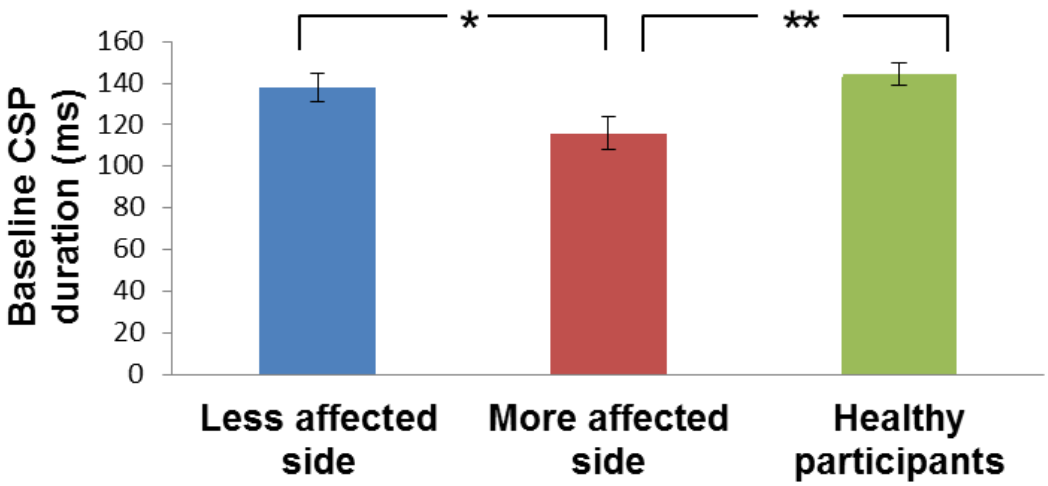


Figure 3.5 Cortical silent period duration at baseline

In PD patients, the CSP is shorter in the hemisphere contralateral to the more affected side compared to the less affected side and also compared to healthy participants. (* $p \leq 0.05$; ** $p \leq 0.01$)

3.4.6 Effect of PAS on baseline corticospinal excitability

There was no within-or between-subject differences in electrical stimuli counting errors during PAS, suggesting equivalent attention levels in different sessions.

The results of the PAS effect on 1mV-MEP amplitude are illustrated in **Figure 3.6**.and **3.7**. Separate 2-way ANOVAs for the APB and the ADM muscles with factors GROUP and TIME revealed that the effect of PAS was different between groups in both APB (GROUP X TIME interaction ($F(6, 135) = 2.6$; $p = 0.02$) and ADM (GROUP X TIME ($F(6, 135) = 3.4$; $p = 0.004$). We explored these interactions further with follow-up ANOVAs in which we made separate comparisons between hemispheres in patients as well as comparisons of each hemisphere with the normal group. The less affected side had a larger response to PAS than the more affected side in both APB (GROUP X TIME ($F(3, 90) = 5.44$; $p = 10^{-3}$) and ADM (GROUP X TIME ($F(3, 90) = 5.55$; $p = 10^{-3}$) muscles. When the less affected side was compared to healthy participants there was no difference between the responses to PAS in the APB muscle; however the less affected side showed a spread of PAS effect to the ADM that was not present in healthy participants (GROUP X TIME ($F(3, 90) = 4.36$; $p = 0.006$). Finally, comparison of the more affected side of PD patients to healthy participants revealed that more affected side had less response to PAS in APB muscle. There was no spread of PAS response to the ADM in the more affected side or

in healthy participants. Within-group effects of PAS were further confirmed in separate ANOVAs for each group and muscle.

Table 3.2 Group comparisons of PAS effect in APB and ADM muscle

	APB	ADM
Less Affected Side Vs. More Affected Side Vs. Healthy participants		
GROUP	n.s.	n.s.
TIME	F (3, 135) =10.1 P<0.0001	n.s.
GROUP X TIME	F (6,135) =2.6 p=0.02	F (6,135) =3.4 p=0.004
Less Affected Side Vs. More Affected side		
GROUP	F (1, 30) =4.42 p=0.04	n.s.
TIME	F (3, 90) =6.05 p=0.0008	n.s.
GROUP X TIME	F (3, 90) =5.44 p=0.001	F (3, 90) =5.55 p=0.001
Less Affected Side Vs. Healthy Participants		
GROUP	n.s.	n.s.
TIME	F (3, 90) =11.1 p=0.0001	n.s.
GROUP X TIME	n.s.	F (3, 90) =4.36 p=0.006
More Affected Side Vs. Healthy Participants		
GROUP	F (1, 30)=5.17 p=0.03	n.s.
TIME	F (3,90) =3.39 p=0.02	n.s.
GROUP X TIME	F (3,90) =2.89 p=0.04	n.s.

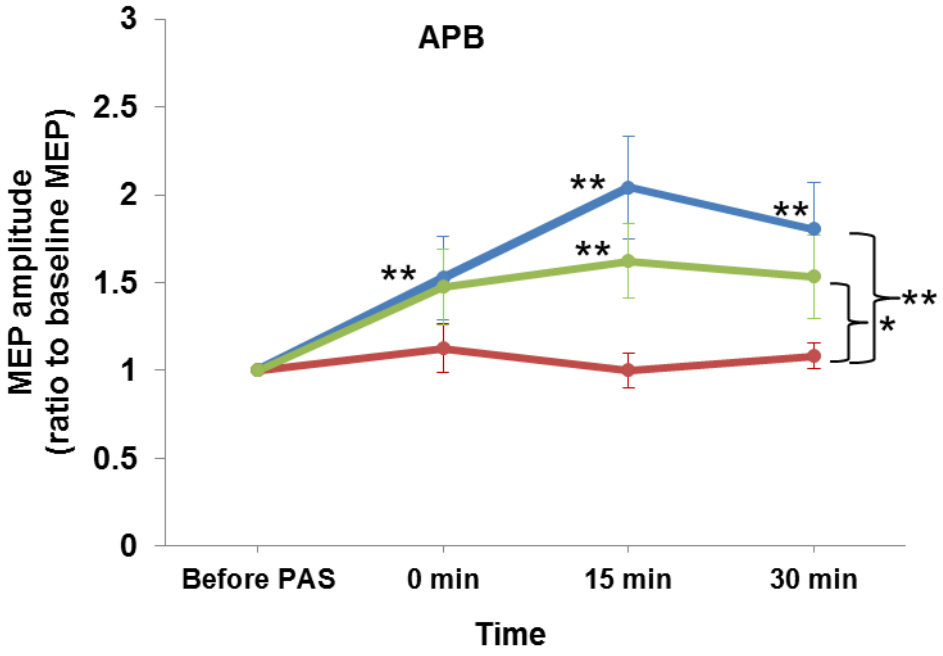


Figure 3.6 PAS effect on corticospinal excitability, as measured by change in 1mV MEP amplitude in APB muscle

In the APB muscle on the less affected side (blue couloured line), PAS increased the 1mv MEP amplitude ($F(3, 45) = 7.19$; $p<10^{-3}$; 1-way ANOVA) at all 3 time points : p= 0.004 at 0 min, $p<10^{-3}$ at 15 min and p= 0.003 at 30 min.There was no significant effect of PAS in APB muscle on the more affected side (red coloured line). In healthy participants (green couloured line) PAS increased 1mv-MEP amplitude in the APB muscle ($F(3, 45) = 4.02$; p=0.01; 1-way ANOVA) only at the 15 min time point.The data is plotted as a ratio to the baseline 1mV-MEP amplitude. Group differences are marked with brackets. (* $p<0.05$, ** $p \leq 0.01$).

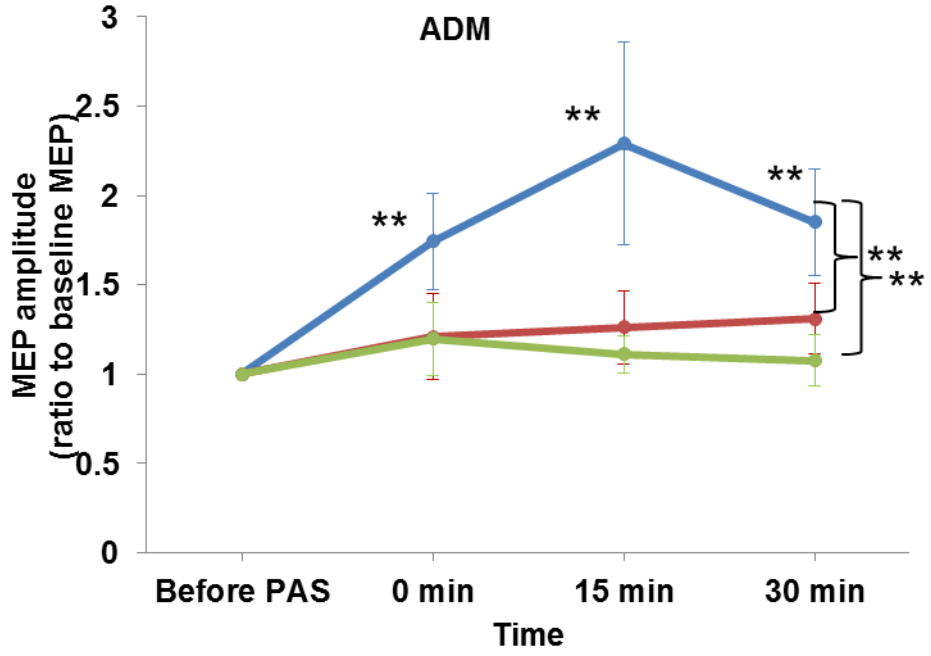


Figure 3.7 PAS effect on corticospinal excitability, as measured by change in 1mV MEP amplitude in ADM muscle.

In the ADM muscle, on the less affected side PAS increased 1mv MEP amplitude at all 3 time points ($F(3, 45) = 6.23$; $p=10^{-3}$; 1-way ANOVA), $p= 0.002$ at 0 min, $p<10^{-3}$ at 15 min and $p= 0.002$ at 30 min .There is no significant effect of PAS in the ADM muscle on the more affected side or in healthy participants.

Correlation analysis between lateralised UPDRS score and average PAS response in APB disclosed that less severe motor symptoms were associated with a greater response to PAS (R= - 0.397; p=0.025) (**Figure 3.8**). There was no difference in our measure of attention during PAS between different TMS sessions or between groups.

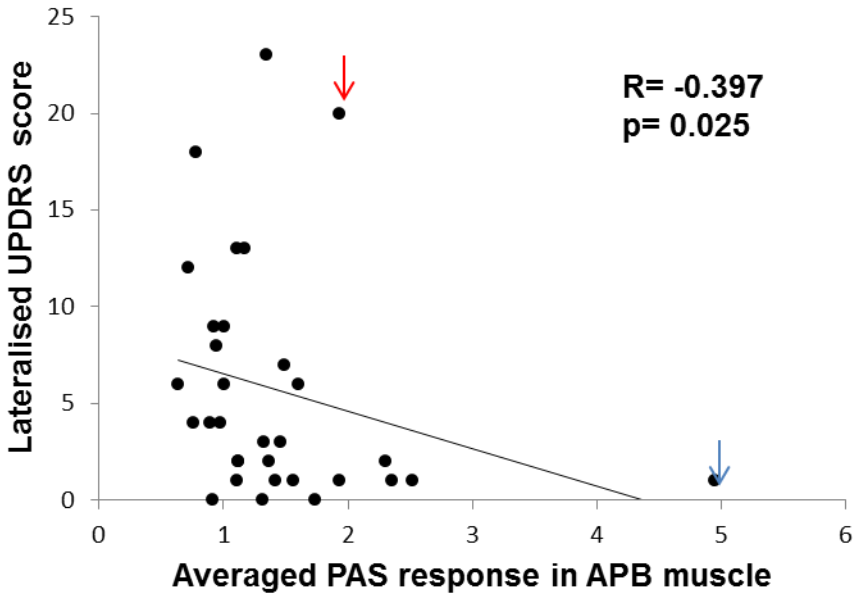


Figure 3.8 Correlation between PAS induced plasticity in APB muscle and the clinical severity of PD

The UPDRS score is associated with a larger response to PAS. Note that the correlation was significant even when the "outlier "indicated by the arrow (blue arrow: less affected side; red arrow: more affected side) is excluded from the analysis (R= - 0.461 p=0.01).

3.4.7 Effect of PAS on CSP

Since the baseline CSP was different between groups, to examine the effect of PAS on CSP, we expressed the duration of CSP at each point after PAS as a ratio to the baseline CSP and computed 2-way ANOVA with factors GROUP (3 levels) and TIME (3 levels: normalised CSP duration at 0,15 and 30 min after PAS). This analysis revealed a difference between groups (factor GROUP ($F(2, 45) = 5.0$; $p = 0.01$) due to an overall stronger effect of PAS on CSP duration in the hemisphere contralateral to the more affected side compared to healthy participants ($p=0.01$). There was no difference in the CSP duration between sides in PD patients or between the hemisphere contralateral to the less affected side in PD and healthy participants.

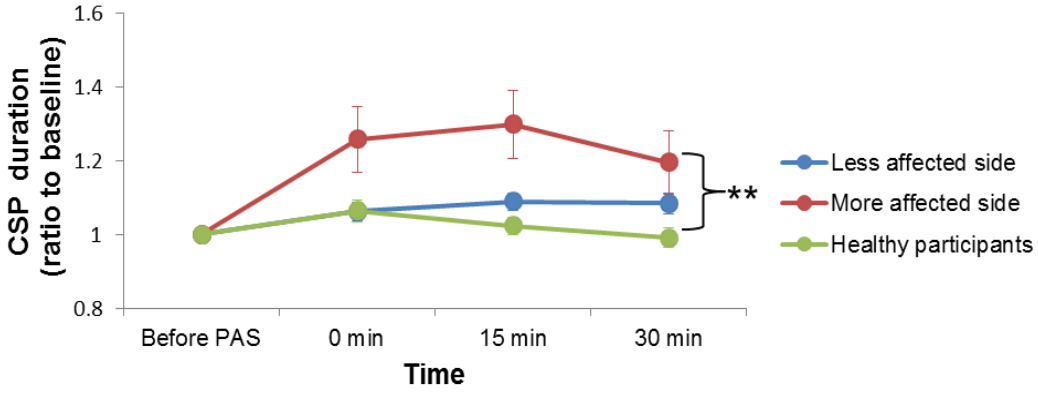


Figure 3.9 The effect of PAS on CSP duration

There is a stronger effect of PAS on CSP duration in the hemisphere contralateral to the more affected side, comparing to healthy participants. (**p ≤0.01)

3.5 Discussion

The main finding of this study was that clinically asymmetric PD patients had a heightened response to a plasticity protocol (PAS) in the hemisphere contralateral to the less affected hemisphere, in contrast to an absent PAS response in the more affected hemisphere. The asymmetry in the electrophysiological findings between the hemispheres was also reflected in intracortical inhibition; the hemisphere contralateral to the less affected side showed preserved SICI and CSP, while in the hemisphere contralateral to the more affected side SICI was reduced and CSP shortened. These asymmetries cannot be explained by differences in the baseline corticospinal excitability, as there were no differences in IO curves and motor thresholds between the two sides.

The absence of the PAS response in the hemisphere contralateral to the more affected side is in line with previous studies in patients with more advanced PD (Morgante et al., 2006, Ueki et al., 2006), who showed a decreased response to PAS in the “off” state that normalised with L-Dopa (Ueki et al., 2006). The reduced response to PAS is explained as being secondary to an abnormal BG output (Obeso et al., 2008) or to result from reduced dopamine at the cortical level (Gaspar et al., 1991). Since there is major bilateral (albeit asymmetric) dopaminergic loss even in early clinically asymmetrical PD (Tissingh et al.,

1998) one might expect a similar reduction of PAS in both hemispheres in the tested patients. On the contrary, we found an increased (compared with healthy age matched subjects) LTP-like plasticity with loss of topographic specificity in the hemisphere contralateral to the less affected side suggesting that there is a functional reorganisation of sensorimotor cortex contralateral to milder signs of PD. These findings may represent a compensatory change or they may reflect disease related maladaptive plasticity. The negative correlation between severity of motor symptoms and the amount of response to PAS suggests that this is a compensatory change.

In health, BG neurons are highly “tuned” to fire in specific circumstances related to different parameters of movement and to contextual cues (Mink and Thach, 1991). There is evidence to suggest that BG dysfunction in PD leads to a loss of specificity of the surviving neurons and their connected structures (Bronfeld and Bar-Gad, 2011). Such changes could alter the precise coupling between sensory inputs and motor outputs that is characteristic of the sensorimotor cortex. Since PAS relies on the interaction between sensory afferents and motor output of the homologous muscle, loss of specificity could lead to spread of facilitation to the ADM muscle on the less affected side. The fact that the more affected side showed no response to PAS, even in the target APB muscle, might be explained by severe dopaminergic deficit in the more affected hemisphere, as seen in more advanced disease. Notably, it has been shown

that healthy subjects have an inverted “U”-shaped dopaminergic dose–plasticity response curve, in which low dopaminergic tone impairs plasticity, while moderate doses facilitate plasticity (Kuo et al., 2008, Monte-Silva et al., 2009) However the nature of such a non- linear relationship, although likely important for understanding our results, has not been specifically investigated in PD.

Another novel finding of the present study is that PD patients had normal SICI in the less affected hemisphere. SICI was absent in the more affected hemisphere, in line with previous findings of reduced SICI in more advanced PD (Ridding et al., 1995a). We used a CS intensity of 90% of RMT to test SICI since this yields the clearest difference between PD and healthy individuals (MacKinnon et al., 2005). Detailed studies assessing the SICI- intensity curve or using different coil orientations indicate that GABAergic inhibitory circuits mediating SICI might be normal in PD while decreased SICI possibly reflects a decreased threshold for intracortical facilitation at higher CS intensities (MacKinnon et al., 2005, Hanajima et al., 2011, Ni et al., 2013). Irrespective of the underlying mechanism, impaired SICI in PD is thought to be related to dopaminergic deficiency since it is normalised with dopaminergic treatment (Ridding et al., 1995a, Pierantozzi et al., 2001). Overall, our results imply that the dopaminergic deficit in the less affected hemisphere may still be under the critical threshold to trigger an impairment of SICI. This would be consistent with a positive correlation between disease severity and reduced SICI.

We found significantly shorter CSP in the hemisphere contralateral to the more affected side and normal CSP in the hemisphere contralateral to the less affected side, confirming previous reports (Cantello et al., 2007). In the present study, the PAS effect on CSP was not statistically different between the two sides in PD patients and was even stronger in the hemisphere contralateral to the more affected side compared to healthy participants. This is in contrast with advanced PD patients “off” dopaminergic treatment (Bagnato et al., 2006, Morgante et al., 2006) and implies that circuits mediating the PAS effect on CSP are preserved in early PD.

A critical question which is highlighted but left unresolved by this current study is whether the alterations in the plasticity response in the hemisphere corresponding to the clinically less affected side represent a beneficial compensatory change that helps prevent motor symptoms progressing or a maladaptive change that reflects disease progression. It might be possible to determine with follow-up of early asymmetric patients if persistence of enhanced plasticity is associated with slower progression of the motor signs on the less affected side, suggesting that this electrophysiological change reflects a beneficial compensatory process, or the converse which would suggest that it reflects a maladaptive process. The change in asymmetry of the PAS response between hemispheres over time could be developed into a electrophysiological marker of disease progression.

Chapter 4 **Changes in sensorimotor cortical plasticity and intracortical inhibition with progression of Parkinson's disease: revealing compensatory mechanisms**

Having demonstrated interhemispheric difference in sensorimotor cortical plasticity in asymmetric PD patients, the following set of experiments sough to answer the critical question of whether or not increased sensorimotor cortical plasticity in the hemisphere contralateral to the less affected side reflects an adaptive, compensatory change that helps prevent motor symptoms emerging or it represents a maladaptive change that heralds the symptom progression. We therefore investigated the relationship between the change in PAS response and the other TMS measures and progression of motor signs, by following up the same patients for a period of 1 year.

The work presented in this Chapter is submitted in Journal of Neurology, Neurosurgery and Psychiatry: Kojovic M, Kassavetis P, Bologna M, Pareés I, Rubio-Agusti I, Beraredelli A, Edwards MJ, Rothwell JC an Bhatia KP. Longitudinal electrophysiological study in Parkinson's disease: revealing compensatory changes

4.1. Summary

The same patients who underwent the experiments presented in Chapter 3 were clinically examined and retested with TMS after 6 and 12 months. On each occasion we measured MTs, IO curves, SICI, ICF and CSP and sensorimotor cortical plasticity using the excitatory PAS protocol. Patients were also rated on the UPDRS scale. We found no difference and no further change in MTs between sides. The IO curve was steeper (with higher intensities) in the hemisphere contralateral to the more affected side and this interhemispheric difference remained constant. The asymmetry in SICI also persisted at 1 year. In contrast, the initially reduced CSP in the hemisphere contralateral to the more affected side subsequently increased, resulting in a side-to-side equalisation of CSP. The interhemispheric differences in sensorimotor cortical plasticity were still present after 1 year. In individual patients, the decline in asymmetry of the CSP and the PAS response was correlated with the reduction in asymmetry of the clinical symptoms. We suggest that an initially reduced CSP in the hemisphere contralateral to the more affected side and increased plasticity in the hemisphere contralateral to the less affected side may be compensatory, preventing clinical expression of disease progression.

4.2. Introduction

In PD motor symptoms emerge after a prolonged period of dompaminergic loss, suggesting there is activity of adaptive or compensatory mechanisms (Zigmond et al., 1990, Hornykiewicz, 1998, Lee et al., 2000, Appel-Cresswell et al., 2010). In the early preclinical stage, adaptive changes occur within the nigrostriatal synapses, and through various mechanisms to keep synaptic dopamine at relatively constant level (Hornykiewicz and Kish, 1987, Zigmond et al., 1989, Zigmond, 1997). With further neurodegeneration, nigrostriatal compensatory mechanisms can no longer keep up with on-going dopaminergic cell loss and BG output structures become further involved in the compensatory process (Bezard et al., 2003, Boulet et al., 2008). Finally, by the time of the emergence of motor signs, compensation presumably engages cerebral circuits *outside* the BG (Bezard et al., 2001, Obeso et al., 2004) including thalamo-cortical connections and the cerebellum (Bezard et al., 2003, van Nuenen et al., 2009, van Nuenen et al., 2012). The role of the sensorimotor cortex in compensation for the dopaminergic deficit has been less well studied (Sabatini et al., 2000). As found in our previous study, presented in Chapter 3, clinical asymmetry of motor signs in early PD is reflected in interhemispheric asymmetry of the electrophysiological measures: the less affected (LA) hemisphere has increased sensorimotor cortical plasticity as measured by the response to PAS25 protocol, while in the more affected (MA) hemisphere plasticity is reduced. A critical question arising from these results is the pathophysiological significance of enhanced plasticity: does increased motor cortical plasticity on the LA

hemisphere reflect an adaptive change that helps prevent motor symptoms emerging or does it represent maladaptive change that heralds disease progression? In the present study we aimed to answer this question by investigating the relationship between change in the PAS response (and other TMS measures) and progression of the motor signs in the same PD patients during a period of one year.

4.3. Methods

4.3.1. Patients

From the 16 drug- naive patients who completed baseline set of experiments described in Chapter 3, 12 patients completed the follow-up study. The remaining four patients were unavailable due to different reasons: one was undergoing treatment for newly diagnosed breast carcinoma, one lived remotely and was not keen to travel the long distance and two decided not to participate further, without giving particular reasons. Subsequent to the baseline set of experiments (for which all patients were drug-naïve), the patients underwent a consultation with a movement disorders specialist and were given all the necessary information about available symptomatic treatments. Because of the ethical issues, patients were not randomised into treated and untreated groups, but it was dependent upon themselves and their treating physician to decide

whether and when to start treatment for PD and with what kind of dopaminergic medications. Irrespective of the on-going treatment, patients were retested at 6 months and 12 months. At the time of follow up, all treated patients had been on a stable dosage of dopaminergic medication for previous the 3 months and were tested in an off-state after overnight withdrawal of treatment (at least 12 hours). Patients' demographic, clinical and treatment data are given in **table 4.**

Table 4.1 Demographic and clinical characteristics of the patients and treatment data

Patient	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***	***Mean (SEM)***
Age	34	44	66	62	48	62	52	71	67	64	50	61	56.7 (3.2)
Sex	F	M	M	F	M	M	M	M	M	M	M	M	
Treatment baseline	/	/	/	/	/	/	/	/	/	/	/	/	
Treatment 6 months	ras 1mg rop 8mg	ras 1mg	ras 1mg	prp 1.5mg	L-Dopa 300 mg	/	/	/	/	/	/	/	
Treatment 12 months	ras 1mg rop 8mg	ras 1mg	ras 1mg	prp 1.5mg	L-Dopa 300 mg	ras 1mg prp 1.5mg	ras 1mg prp ER 0.375 mg	ras 1mg L-Dopa 300 mg	L-Dopa 300 mg	rotg 6mg	/	/	
UPDRS LA baseline	1	2	1	0	1	0	1	1	1	2	7	2	1.58 (0.42)
UPDRS LA 6 m	1	0	3	1	1	1	2	1	2	4	11	4	2.58 (0.53)
UPDRS LA 12 m	2	1	2	2	1	4	3	5	2	3	9	6	3.33 (0.37)

Cont.

UPDRS MA baseline	18	8	5	12	11	9	6	10	4	14	18	3	9.83 (0.46)
UPDRS MA 6 m	13	11	8	10	13	10	7	10	4	17	15	5	10.25 (0.35)
UPDRS MA 12 m	21	8	7	13	18	8	8	10	4	15	15	9	11.33 (0.43)
UPDRS index baseline	0.89	0.60	0.67	1	0.83	1.00	0.71	0.82	0.60	0.75	0.44	0.2	0.71 (0.08)
UPDRS index 6 m	0.86	1.00	0.45	0.82	0.86	0.82	0.56	0.82	0.33	0.62	0.15	0.11	0.62 (0.11)
UPDRS index 12 m	0.83	0.78	0.56	0.73	0.89	0.33	0.45	0.33	0.33	0.67	0.25	0.20	0.53 (0.1)
UPDRS total baseline	24	12	9	12	18	10	8	18	9	26	33	5	15.33 (0.63)
UPDRS total 6months	17	14	17	13	20	12	9	24	10	34	31	9	17.5 (0.85)
UPDRS total 12 m	26	12	14	16	24	18	17	20	10	28	38	22	20.42 (0.5)

Abbreviations: F, female; M, male ras,rasegiline; rop,ropinirol; prp, pramipexole; prp ER, pramipexole extended release; UPDRS LA, UPDRS on the less affected side; UPDRS MA, UPDRS on the more affected side; UPDRS total, total motor UPDRS

4.3.2. Clinical measures

Clinical disease severity was assessed with the motor section of the MDS-UPDRS scale (Goetz et al., 2008b). For the less and more affected side, lateralised UPDRS scores were calculated as the sum of the items for bradykinesia, rigidity and tremor (items 3.3 to 3.8 and 3.15 to 3.17) for each side. To express the severity of motor symptoms and their distribution between sides within one measure we calculated an UPDRS asymmetry index, given as: (MA-LA)/ (MA + LA), where MA and LA represent lateralised UPDRS score from more and less affected side respectively (Espay et al., 2005, Li et al., 2007). The UPDRS asymmetry index ranges from 1 when symptoms are only on one side (i.e. complete asymmetry of symptoms) to 0, when symptoms are evenly distributed.

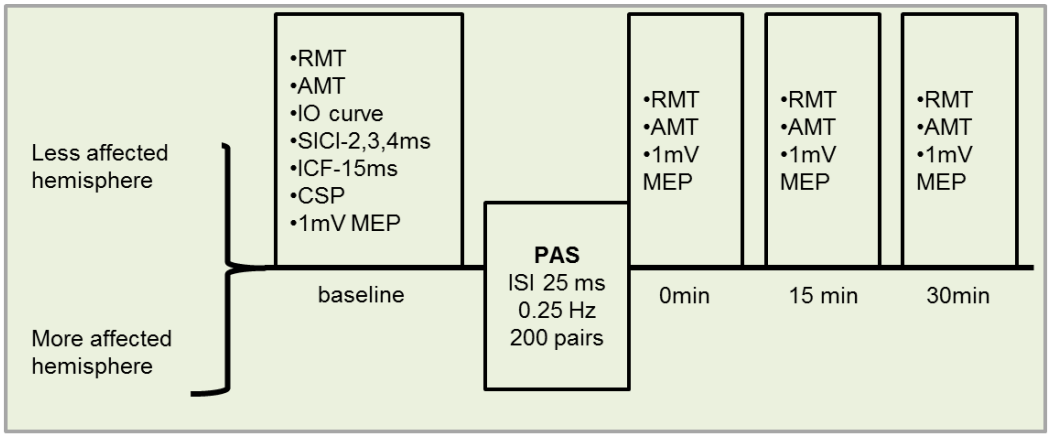
4.3.3. EMG recordings and TMS

We used exactly the same methods as performed for the baseline set of experiments (chapter 3).

4.3.4. Experimental design

At baseline, 6 months and 12 months patients were tested on both hemispheres, corresponding to the less and more affected side in different TMS

sessions, separated by 1 week (**Figure 4.1**). Patients were always studied at the same time of the day, during morning hours. The motor assessment with the UPDRS scale was performed before the TMS testing. The order of the tested hemisphere was balanced between subjects. In each session we first measured RMT, AMT, 1mV MEP, IO curve, SICI, ICF and CSP. Motor cortex plasticity was probed using PAS25. We assessed the effect of PAS25 on 1mV MEPs amplitude at 3 time points after PAS: 0, 15 min and 30 min.



- **Baseline: all patients drug naive**
- **6 months: 5 treated, 7 untreated**
- **12 months: 10 treated, 2 untreated**

Figure 4.1 Follow up study design

4.3.5 Statistical analysis

Since all patients were tested “off” dopaminergic medications, we began our analysis by considering treated and untreated patients together. Friedman ANOVAs were used to assess changes in the motor total UPDRS score, the lateralised UPDRS score and the UPDRS index over time (baseline, 6 months, and 12 months). To test the changes in electrophysiological measures we used ANOVAs with factors TIME and SIDE (for RMT, AMT, CSP and ICF) or ANOVAs with additional within-subjects factors depending on the variable tested. For changes of IO curves factor STIMULUS INTENSITY (10 levels of stimulator output intensity) was used. For analysis of SICI, for each ISI, conditioned MEP amplitude was averaged and normalized to average unconditioned MEP amplitude and entered into ANOVA with factors: SIDE, TIME and ISI (2,3, and 4 ms). For analysis of the PAS effect, for each time point after PAS the amplitude of the MEP was averaged and normalized to the average MEP amplitude before PAS and entered into ANOVA with factors: TIME, SIDE, MUSCLE (APB and ADM) and TIME POINT AFTER PAS (0 , 15 min and 30 min after PAS). In addition, we expressed the interhemispheric difference in electrophysiological measures between the LA and MA side as the ratio between sides and entered it in ANOVA with factor TIME.

In a secondary analysis, we compared treated and non-treated PD patients. At 6 months, we used ANOVA with the factor GROUP (treated vs. untreated) as a

between-subject factor and factor TIME (baseline vs. 6 months) and SIDE as within subjects factor. At 12 months, the majority of patients were treated and the difference between treated and untreated could not be assessed due to the small number of untreated patients. We however assessed if there were differences between “early” vs. “late” treated patients. Early treatment refers to initiation of treatment within 1 month of the baseline set of experiments (5 patients), while late treatment refers to initiation of treatment after the second set of experiments (5 patients) or no treatment at all at the time of 3rd set of experiments (2 patients).

To determine the relationship between changes in motor scores and the electrophysiological measures over the all three longitudinal time points we computed within-subject correlation coefficients (Bland and Altman, 1995) using the UPDRS index as an outcome variable, while subjects and the interhemispheric ratio of the electrophysiological measure of interest (PAS ratio, SICI ratio or CSP ratio) were predictor variables. Statistical analysis was done on the raw data. However, to plot the correlation figures, data at each time point were first expressed as a difference (delta) between measurement at that time point and patient‘s average of all 3 time points and this value was then expressed as a fraction of the individual patient's average, using the following computation : (CSP ratio $_{\text{baseline, 6 or 12 months}}$- average CSP ratio) / average CSP ratio or (PAS ratio $_{\text{baseline, 6 or 12 months}}$- average PAS ratio) / average

PAS ratio; and (UPDRS index $_{\text{baseline, 6 or 12 months}}$- average UPDRS index) / average UPDRS index.

4.4. Results

4.4.1. Clinical measures

Results on changes in clinical measures are illustrated in **Figure 4.2**. As noted in the **table 4. 1**, none of the patients were taking any anti-parkinsonian medication at baseline, five had started treatment at 6 months and 10 of the 12 were treated at 12 months. As expected, the patients' practically defined "off" state deteriorated gradually over time. A Friedman ANOVA on total UPDRS score revealed a significant effect of TIME ($\chi 2$ =12.6; p =0.002) which was due to the score being higher at 12 months compared to baseline (p=0.003), while the difference between baseline vs. 6 months and 6 vs. 12 months just failed to reach significance (p=0.06 and p=0.09, respectively). Breaking down the data into results from the LA and MA sides showed that although the scores increased on both sides, this was significant only on the LA side (χ^2 =10.6; p =0.005), due to the score being higher at 12 months compared to baseline (p=0.007), while there was no significant difference between 6 months and baseline (p=0.06) or 6 vs. 12 months (p=0. 2). For MA side, there was no significant change in the UPDRS score over time (χ^2 =3.3; p =0.2). Despite changes in the lateralised scores, the side difference in symptom severity persisted, with the MA side having higher UPDRS at all 3 time points (Wilcoxon

sign rank test: p = 0.002 at baseline, p = 0.002 at 6 months and p = 0.002 at 12 months).

Overall, the UPDRS asymmetry index fell over the 12 month period (χ^2 =3.3; p =0.01). It was smaller at 12 months compared to baseline (p=0.03), while there was no difference between baseline and 6 months or 6 and 12 months (p=0.06 and p=0.4, respectively).

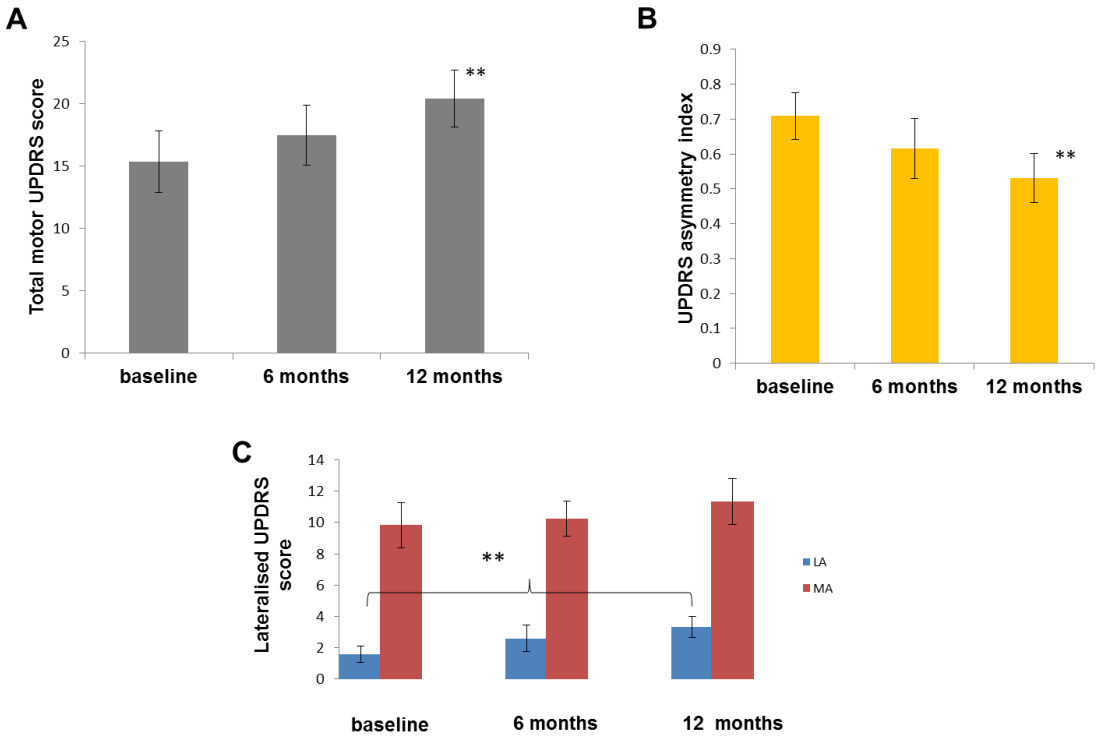


Figure 4.2 Change in clinical measures

(A) total motor UPDRS score , (B) UPDRS asymmetry index and (C) lateralised UPDRS score over 12 months period. (**p<0.01).

4.4.2. Baseline corticospinal excitability

For RMT and AMT, ANOVA revealed no significant effect of the factors SIDE or TIME and no significant SIDE x TIME interactions, indicating there were no differences in motor thresholds between sides and no change over time. For IO curves, a 3-way ANOVA revealed significant effect of SIDE ($F(1, 11) = 6.08$; $p = 0.04$) and SI ($F(9, 99) = 52.7$; $p < 10^{-3}$), and significant SIDE X SI interaction ($F(9, 99) = 2.65$; $p = 0.008$). This was due to higher MEPs on the MA side with SI: 150%, 160% and 170% RMT ($p = 0.04$, $p = 0.03$ and $p = 10^{-3}$, respectively). The factor TIME was not significant. These results indicate that the IO curve was steeper on the MA hemisphere at all 3 assessments (**Figure 4.3**).

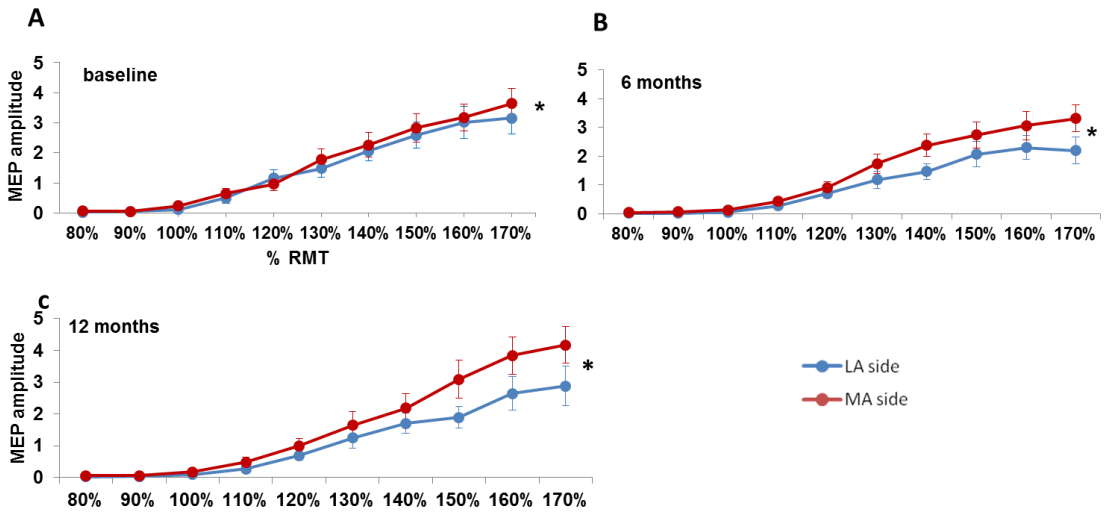


Figure 4.3 IO Curve- change over 12 months

The mean MEP amplitude (±SEM) is given on the y-axis against the stimulus intensity given on the x-axis (as a percentage of RMT stimulus intensity). There is an interhemispheric difference in IO curve: at higher stimulus intensity IO curve is steeper in the hemisphere contralateral to the more affected side comparing to the hemisphere contralateral to the less affected side. This difference between sides is evident at baseline, and even more so at 6 months and 12 months.

4.4.3. SICI

Like the IO curves above, SICI was less effective in the MA hemisphere but did not change over the 12 month assessment period. A 3-way ANOVA showed significant effects of SIDE ($F(1, 11) = 11.1$; $p = 0.006$) and ISI ($F(2, 22) = 10.51$; $p < 10^{-3}$) due to the fact that there was less effective SICI on the MA hemisphere and greater SICI at ISIs 2 ms and 3 ms compared to 4 ms ($p < 10^{-3}$ and $p = 0.01$, respectively). There was no effect of TIME nor any 2-way or 3-way interactions (**Figure 4.4**). There was no correlation between changes in SICI interhemispheric ratio and changes in UPRDS asymmetry index.

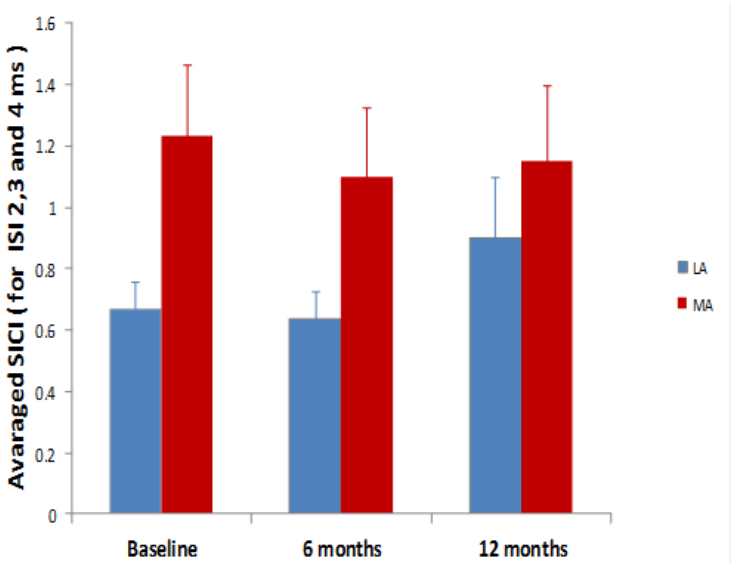


Figure 4.4 Short Interval Intracortical Inhibition—change over 12 months

There is an interhemispheric difference in SICI, evident at baseline, at 6 months and at 12 months.

4.4.4. ICF

There was no significant effect of factors SIDE and TIME and no significant SIDE x TIME interaction, indicating no differences in ICF between sides and no change over time (**Figure 4.5**).

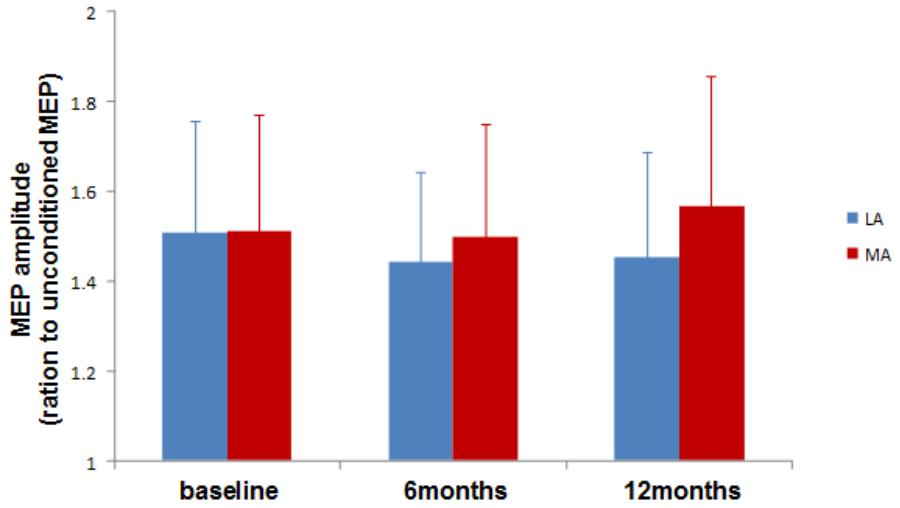


Figure 4.5 Intracortical Facilitation— change over 12 months

There is no significant interhemispheric difference in ICF (ISI 15 ms) and no significant change over time. Data is plotted as a ratio to the unconditioned MEP amplitude.

4.4.5. CSP

ANOVA revealed no significant effect of the factors TIME or SIDE. However there was significant TIME X SIDE interaction ($F(2, 22) = 5.42$; $p=0.01$) due to the fact that the CSP in the MA hemisphere was longer at 6 and 12 months compared to baseline ($p=0.02$ and $p= 0.02$, respectively). Thus, an initially shorter CSP in the MA hemisphere increased at 6 months, resulting in equalisation of the CSP between sides (**Figure 4.6**). There was no change on the LA hemisphere. Within-subject correlation analysis revealed that the reduced difference between the CSP duration in the two hemispheres was correlated with the decrease in UPDRS asymmetry index ($r=0.5$, $p=0.02$) (**Figure 4.7**)

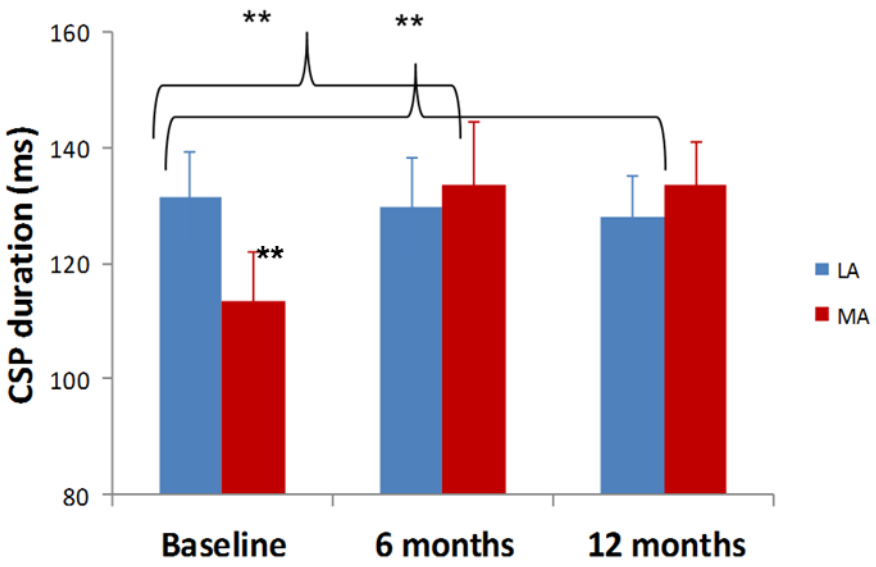


Figure 4.6 Cortical silent period— change over 12 months

CSP duration: At baseline, CSP is shorter in the more affected hemisphere compared to the less affected hemisphere. After 6 months CSP increased in the more affected hemisphere , while there was no significant change in CSP duration in the less affected hemisphere , resulting in equalisation in CSP between sides at 6 and 12 months(**p<0.01)

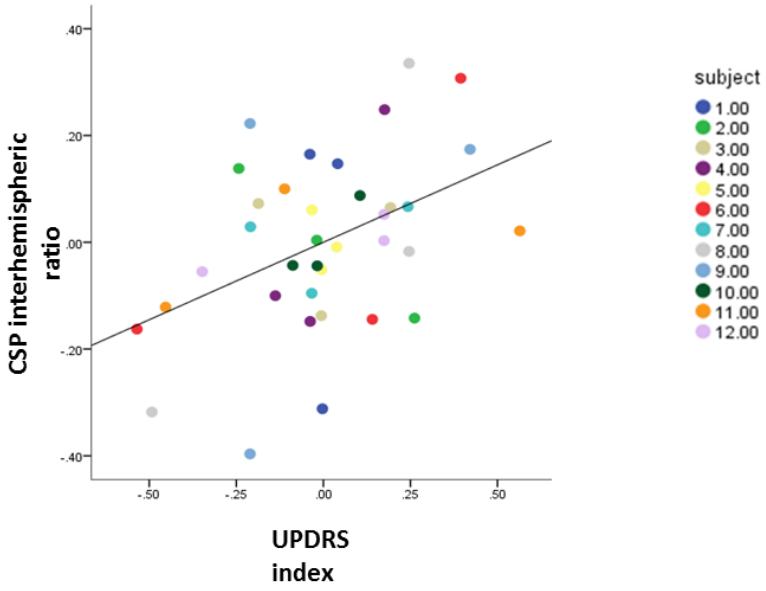


Figure 4.7 Within-subjects correlation between change in UPDRS asymmetry index and change in CSP interhemispheric ratio

This correlation shows that a decrease in CSP interhemispheric ratio is associated with decrease in UPDRS asymmetry index, thus with progression of motor signs. The black line indicates the overall correlation for the whole group. Different colours correspond to different patients and each patient is represented with 3 points (for baseline, 6 and 12 months measurements).

4.4.6 PAS

There was no within-or between-subject difference in electrical stimuli counting errors during PAS, thus excluding differences in attention levels in different sessions.

For PAS response, ANOVA revealed significant effect of the factor SIDE ($F(1, 11) = 34.0$; $p<10-3$) due to the larger response on the LA hemisphere. Factors TIME, MUSCLE and TIME POINT AFTER PAS and all interactions between main factors were not significant. These results indicate that the asymmetry of the PAS response between sides detected at baseline was still present at 6 and 12 months (**Figure 4.8**).

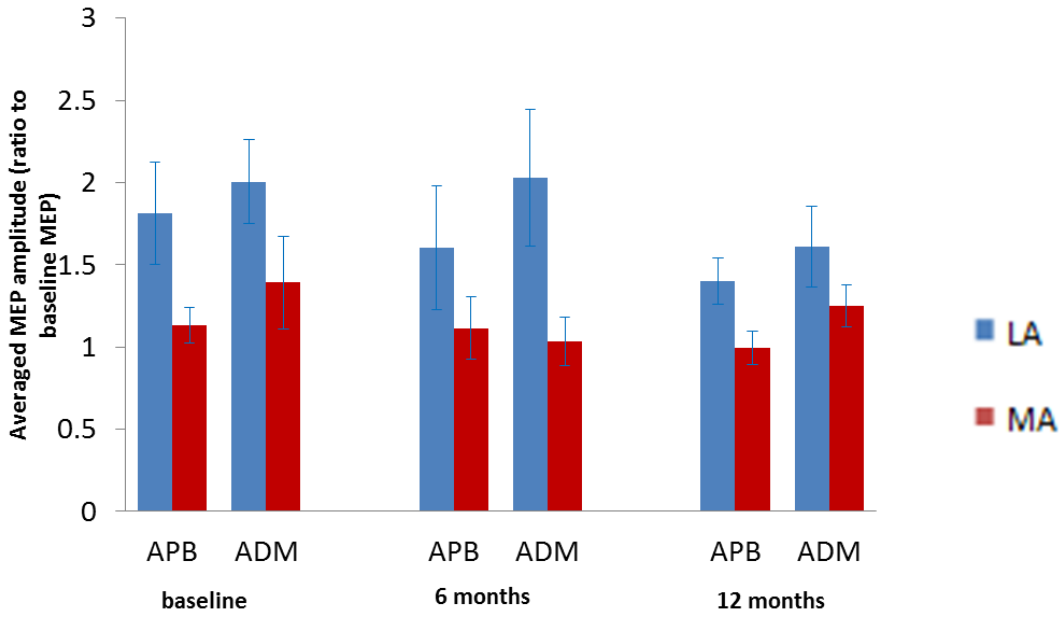


Figure 4.8 Change of PAS response

The interhemispheric difference in PAS response (expressed as an averaged PAS response for 0, 15 and 30 min after PAS) is evident at baseline, at 6 months and at 12 months, in both APB and ADM muscle.

However there were substantial inter-individual differences in how PAS changed over time. Within individual patients, a decrease in the PAS interhemispheric ratio was associated with decrease in UPDRS asymmetry index (**Figure 4.9**).There was a positive within-subjects correlation between APB interhemispheric (and ADM interhemispheric ratio) and UPDRS asymmetry index (APB: r=0.51, p=0.03 and ADM: r=0.43, p=0.03).

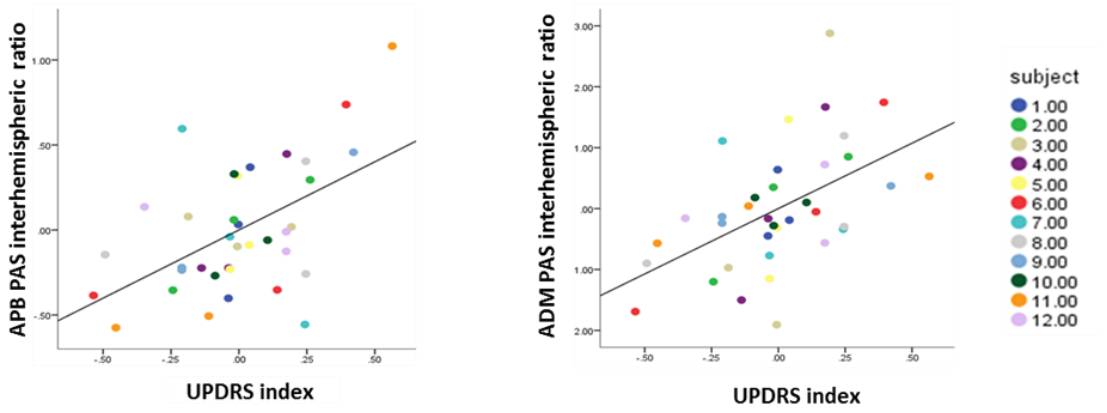


Figure 4.9 Within-subjects correlation between change in UPDRS asymmetry index and change in PAS interhemispheric ratio.

This correlation shows that the decrease in PAS interhemispheric ratio in both APB and ADM muscles is associated with a decrease in the UPDRS asymmetry index, and thus with progression of the motor signs. Black lines indicate the overall correlation for the whole group.

4.4.7 Treated vs. untreated patients and Early vs. Late treated patients

For all clinical measures and all TMS measures there was no difference between treated and untreated patients or between early and late treated, as indicated by no significant effect of the factor GROUP and no interactions of GROUP with TIME in any of the analysis.

4.5. Discussion

This is the first longitudinal study that was design to address changes in different electrophysiological measures with the progression of early PD. In the first year after initial presentation, there was a gradual decrease in clinical asymmetry. Despite the clinical deterioration, there was no overall change in: motor thresholds to TMS, IO curves of MEP recruitment; GABAa inhibition measured in the SICI protocol, ICF; or synaptic plasticity of sensorimotor cortex measured using the PAS protocol. Initial asymmetries in IO curves, SICI and PAS persisted between the less and more affected hemispheres. The only physiological measure that changed was the GABAb mediated CSP, which was shorter in the LA hemisphere at onset but became equalised between the two sides after 6 months and remained the same after 1 year. Although the sample was small, there was no difference between “early” and “late” treated patients, similar to previous physiological work that suggested that there are no long-term

modifying effects of L-Dopa on electrophysiological measures (Kacar et al., 2012).

The apparent lack of change in the mean data, however, conceals a variation in the rate of progression in individual patients. When we expressed the clinical scores conventionally as an asymmetry index, which normalises the between-side differences to the total score on both sides, there was a significant correlation between the reduction in clinical asymmetry and the corresponding reductions in the asymmetry of CSP and PAS response.

Changes in intracortical inhibition and facilitation

Pharmacologically, short-interval intracortical inhibition is primarily mediated by GABAa receptors and the cortical silent period is subserved by GABAb receptors (Ziemann, 2004, Di Lazzaro et al., 2006). Our results on SICI and CSP suggest that these circuits are differently affected during the course of PD. While the interhemispheric difference in SICI detected at baseline persisted after 6 and 12 months, the initial asymmetry of the CSP between hemispheres subsequently disappeared: the shorter CSP on the MA side increased towards values for the LA side at 6 months. Interestingly, a decrease in the interhemispheric CSP ratio correlated with a decrease in the UPDRS asymmetry index. Thus an initially shorter CSP in the MA hemisphere may reflect a compensatory change serving to facilitate the movement on the side most affected by symptoms. Prolongation of CSP could also be interpreted as

an effect of dopaminergic treatment, but this seems unlikely given that the same change occurred in both treated and untreated patients.

There was no side difference in ICF and no change over 1 year. Many previous studies (Ridding et al., 1995a, Berardelli et al., 1996, Strafella et al., 2000, Kacar et al., 2012) showed normal ICF in patients with more advanced PD, which together with our results suggest that there is no major involvement of the interneurons mediating ICF during the course of PD.

Changes in sensorimotor cortical plasticity

We described in Chapter 3 that early PD patients have an enhanced response to PAS25 on the LA side while the response to PAS25 is absent on the MA side. In the present study, we demonstrate that the interhemispheric difference in sensorimotor cortical plasticity still persists after 1 year. However, this conceals a large variability between patients. In some patients, the asymmetry in PAS response between hemispheres declined whereas there was little change in others. Overall, the rate of change in PAS asymmetry correlated well with the change in asymmetry of the UPDRS: the greater the reduction in PAS asymmetry, the greater the reduction in the asymmetry of the UPDRS. Notably, asymmetry in IO curves is unlikely to be the cause of an asymmetric PAS response in our patients, because if this were the case, we would expect a greater response to PAS on the side with the steeper IO curve, i.e. the MA side (Rosenkranz, 2010). We found the opposite pattern.

Therefore our results challenge the classical view of a generally reduced sensorimotor cortical plasticity in PD. Apart from one study (Bagnato et al., 2006), there is a general consensus that the response to PAS25 is reduced or absent in patients tested “off” medications (Morgante et al., 2006, Ueki et al., 2006, Kacar et al., 2012, Kawashima et al., 2013, Kishore et al., 2013, Udupa and Chen, 2013). However, all these studies dealt with patients who had more advanced PD compared to our patients. Our present results show that motor cortex plasticity can change from being increased early in the course of disease (as it was in the LA hemisphere of our patients), to a complete loss of plasticity response (as in the MA hemisphere in the present patients). We suspect that the two sides in our patients will reach a similar pattern of electrophysiological abnormalities with further disease progression.

Our hypothesis is that the interhemispheric difference in sensorimotor cortical plasticity in early PD represents an adaptive rather than maladaptive change. The increased plasticity at, and perhaps prior to clinical onset may allow cortical areas to compensate in some way for the underlying deterioration in BG output. A possible role of interhemispheric interaction in compensation for dopaminergic deficit has been proposed by Blesa et al.(Blesa et al., 2011), based on the 18F-DOPA PET study of asymmetric PD patients and experiments on MPTP lesioned monkeys. It was proposed that a higher asymmetry of nigrostriatal dopamine deficit, as defined by the relatively

preserved dopaminergic innervation in the less-affected compared to the more affected striatum, might relate to a better compensatory capacity, resulting in higher thresholds for the appearance of motor symptoms on the clinically less affected side. Our study also supports the role of asymmetric hemispheric reorganisation in compensation for PD, although it cannot address the mechanism through which this might be occurring.

Limitations of the study

There are some interpretational issues I would like to mention. The association between change in interhemispheric difference in cortical plasticity and clinical progression is not necessarily sufficient to confirm a direct relationship. Thus, we cannot exclude the existence of some third factor that could have influenced both variables independently. This however seems less likely in the light of a number of previous studies showing the relationship between various excitatory plasticity protocols that work by inducing LTP-like changes at cortical synapses, and improvement in the motor symptoms of PD (Khedr et al., 2003, Khedr et al., 2006, Lomarev et al., 2006).

We found no significant difference between treated and untreated patients or between early and late treated patients in clinical or electrophysiological measures, suggesting that early initiation of dopaminergic treatment does not affect the electrophysiological changes that occur with disease progression.

However, there are limitations to be considered. First, the present study was not primarily aimed to assess the effect of treatment, considering the small number of patients in each group and the relatively short follow-up period. Therefore, subtle differences could have been missed and we cannot exclude that a potentially modifying effect of early dopamine replacement would become apparent after a longer follow- up period. However, this is not the first time that no differences between treated and non-treated patients have been identified. Kacar et al. (2012) also found no clinical differences and no differences in TMS measures, including PAS response, between PD patients who have never been treated with dopaminergic medications and the group of treated patients with similar disease duration, arguing against a disease modifying effect of early treatment.

Finally, as we used as measures of clinical progression a change in lateralised UPDRS score and UPDRS index, that both take into account 3 cardinal motor signs (i.e. rigidity, bradykinesia and tremor) together, we cannot comment if predominant presence of one of the motor signs is associated with specific electrophysiological changes in the sensorimotor cortex. This could be addressed in future studies with a large enough sample of PD patients.

Conclusion

Changes in motor cortex plasticity and GABAb intracortical inhibition are likely sensitive electrophysiological measures of clinical progression in PD. Enhanced cortical plasticity and shortening of the CSP probably reflect compensatory

processes in early PD, which might be relevant for potential therapeutic interventions using non-invasive brain stimulation techniques.

Chapter 5 **Modulation of sensorimotor cortical plasticity with Botulinum Toxin injections in primary dystonia**

Abnormal brain plasticity is thought to play a major role in pathophysiology of primary dystonias. In particular, it has been shown that sensorimotor cortical plasticity is increased as measured by response to a PAS protocol that relies on the integration of sensory input and motor output. In the following set of experiments, we investigated if modification of the afferent input by botulinum toxin (BT) injections may affect sensorimotor cortical plasticity.

The work presented in this Chapter is published in the form of a research article: Kojovic M, Caronni A, Bologna M, Rothwell JC, Bhatia KP, Edwards MJ. Botulinum toxin injections reduce associative plasticity in patients with primary dystonia. *Mov Disord*. 2011 Jun;26(7):1282-9.

5.1 Summary

BT injections ameliorate dystonic symptoms by blocking the neuro-muscular junction and weakening dystonic contractions. We investigated whether BT injections in dystonia patients also affect the integrity of sensorimotor cortical plasticity, one of the key pathophysiological features of dystonia. We applied

rPAS protocol, known to induce LTP-like changes in the primary motor cortex hand area (Quartarone et al., 2006a) to 12 patients with cervical dystonia before, 1 and 3 months after BT injections to the neck muscles. M1 excitability was probed by measuring TMS-evoked MEPs before and after rPAS. We also measured IO curve, SICI, ICF, short afferent inhibition (SAI) and long afferent inhibition (LAI) in the hand muscles and the clinical severity of the dystonia. We found that before BT, rPAS significantly facilitated MEPs in hand muscles. 1 month after injections this effect was abolished, with partial recovery after 3 months. There were significant positive correlations between the facilitation produced by rPAS and (i) the time elapsed since injections of BT and (ii) the clinical dystonia score. One effect of BT treatment is to modulate the afferent input from the neck and we propose that the subsequent reorganisation of the motor cortex representation of the hand muscles may explain the effect of BT on motor cortex plasticity.

5.2 Introduction

The lack of inhibition at multiple CNS levels (Berardelli et al., 1985, Nakashima et al., 1989, Ridding et al., 1995b) and abnormal cortical plasticity contribute to the pathophysiology of dystonia (Quartarone et al., 2003, Edwards et al., 2006). In dystonia, enhanced sensorimotor cortical plasticity extends beyond the clinically affected region and may be detected in the unaffected upper limbs of patients with cervical dystonia (Quartarone et al., 2008). BT inhibits

acetylcholine release from α -motoneurons and is used as an effective treatment for different forms of focal and segmental dystonia. Although the clinical improvement roughly parallels weakness, it is commonly observed that the clinical benefit seems out of proportion to the weakness caused by the injections, suggesting an additional, possibly central effect of BT (Priori et al., 1995, Trompetto et al., 2006). The effects of BT in dystonia have been addressed in several studies. (Priori et al., 1995, Byrnes et al., 1998, Gilio et al., 2000, Thickbroom et al., 2003, Quartarone et al., 2006b, Trompetto et al., 2006). For example, the tonic vibration reflex in patients with writer's cramp is suppressed to a greater extent than maximal voluntary contraction (MVC) and maximum M wave amplitude (M-max) after BT injections and this effect persists even when MVC and M-max returned to baseline, even though some patients still experienced some benefit from the injections (Trompetto et al., 2006). BT treatment normalizes reduced spinal reciprocal inhibition (Priori et al., 1995) and reduced intracortical inhibition (Gilio et al., 2000). Abnormal cortical hand representations revert to normal in patients with focal limb or cervical dystonia after BT injections (Byrnes et al., 1998, Thickbroom et al., 2003). BT has also been shown to reduce the abnormally enhanced plasticity of the trigeminal blink reflex in patients with blepharospasm (Quartarone et al., 2006b). These effects have all been explained in part by a change in the Ia afferent input from muscle spindles caused by BT (Filippi et al., 1993, Rosales et al., 1996).

In the present study, we hypothesized that BT injections may change the response to an experimental cortical plasticity protocol, which is known to be enhanced in primary dystonia (Edwards et al., 2006, Weise et al., 2006). We studied the response to PAS in patients with cervical dystonia (CD) (with or without arm involvement) before, 1 month and 3 months after BT injections into the neck muscles.

5.3 Methods

5.3.1 Participants

We studied 12 patients (8 women, mean age: 53 years; range: 30-72 years) with clinically definite primary CD. Six patients had pure focal CD and 6 patients had CD with mild arm involvement (4 writing dystonia and 2 dystonic arm tremor) that did not require treatment. For the clinical assessment of dystonia we used the Burke-Fahn-Marsden (BFM) scale in order to capture the additional arm involvement in CD and any possible change with BT injections to the neck muscles. The mean duration of the disease was 14 years (range: 3-30 years) and all but one patient was chronically treated with BT type A (Dysport, Ipsen, UK), for an average of 7 years (range: 1-21 years). BT was injected solely into the cervical muscles with a dose ranging from 325 to 850 IU (mean dose 487 IU). None of the patients had ever had injections into their arm muscles. At the time of the study, no patient was receiving medication that we believe could affect the measures performed. Clinical and demographic data are given in **Table 5.1**. All patients were the right-handed.

Table 5.1 Clinical features of patients with primary dystonia

Patients	Age	Disease duration(yrs)	BT therapy duration(yrs)	BT (IU)	BFM pre BT	BFM 1 month	BFM 3 months	Time from last	Muscles injected injected
1*	56	7	3	480	4	4	6	13	spl;trp
2	72	13	10	500	8	1	0.5	12	spl;scm;ls
3	52	5	3	425	4	2	4	18	spl;scm;ls
4*	42	28	8	500	4	2	2	16	spl.scm.tr
5	30	3	1	325	4	2	4	16	spl;scm
6*	65	30	21	850	4	0	2	18	spl;scm
7	37	8	6	425	4	2	2	15	spl;scm;tr;ls
8*	61	26	0	440	2	2	2	/	spl
9	55	21	20	500	6	4	4	37	spl;scm;ls
10*	51	14	2	350	2	2	4	21	spl;scm
11	61	13	9	450	4	2	4	23	spl
12*	58	3	2	600	6	4	6	16	spl;scm;ls;tr
Mean	53	14	7	487	4.3	2.2	3.4	17	

Abbreviations: yrs, years; wks, weeks; IU, international units; BFM, Burke-Fahn-Marsden scale; spl, splenius; scm, sternocleidomastoid; ls, levator scapulae; tr, trapezius. Asterisks (*) indicate patients affected by cervical dystonia with arm involvement: patients 1and 6- dystonic tremor: patients 4, 8, 10, 11- writing dystonia.

5.3.2 EMG recordings and TMS

EMG recordings were made from the APB and FDI muscles. Single and paired pulse TMS of the left primary motor cortex was applied as indicated in Chapter 2. The IO curve was assessed using SI from 70 to 130 % RMT. The SICI and ICF were probed at ISI of 2 and 12 ms, respectively.

Short-latency afferent inhibition (SAI) and long-latency afferent inhibition (LAI) were assessed according to the protocol by Tokimura et al.(2000).For the electrical median nerve stimulation, the intensity was set just above the threshold for evoking a visible twitch of the thenar muscles (approximately three times perceptual threshold). The intensity of the TMS was set to evoke 1mV-MEPs. SAI was probed at the ISI of 25 ms. At this ISI, in healthy subjects, SAI is present and may be modified by rPAS (Quartarone et al., 2006a). LAI was probed at ISIs of 200 ms. For both SAI and LAI, the electrical stimulus preceded the TMS stimulus.

rPAS protocol was delivered using Magstim Rapid² stimulator (Magstim Company, Carmarthenshire, Wales, UK), as described by Quartarone et al. (2006a).The protocol consisted of 600 pairs of median nerve and TMS stimuli continuously delivered on the APB hot spot of the left hemisphere, at a rate of 5 Hz. Each TMS stimulus was preceded by an electrical conditioning stimulus given to the right median nerve at ISI of 25 ms. The median nerve was

stimulated at the wrist using standard bar electrodes with the cathode positioned proximally. The median nerve electrical stimulation was performed with constant current square wave pulses with a pulse width of 500μs. The intensity for the median nerve stimulation was 200% of the perceptual threshold. The intensity of TMS was individually adjusted to 90% of the AMT.

5.3.3 Experimental design

All subjects were studied in three sessions: before BT injections, 1 month after 3 months after BT injections **(Figure 5.1)**. In all subjects, at least 3 months elapsed between the previous injections and the first experimental session (**Table 5.1**). Before each session, patients underwent a clinical assessment with the BFM scale.

In each session the TMS parameters (RMT, 1mV-MEP amplitude, SICI, ICF, SAI and LAI) were measured at four time points: before rPAS, immediately after rPAS (0 min, 30 minutes and 60 minutes after rPAS. Before rPAS in each of three experimental sessions, we also measured the IO curve in 7 steps, using the TMS intensity from 70 to 130% of the 1mV-MEP thresholds.

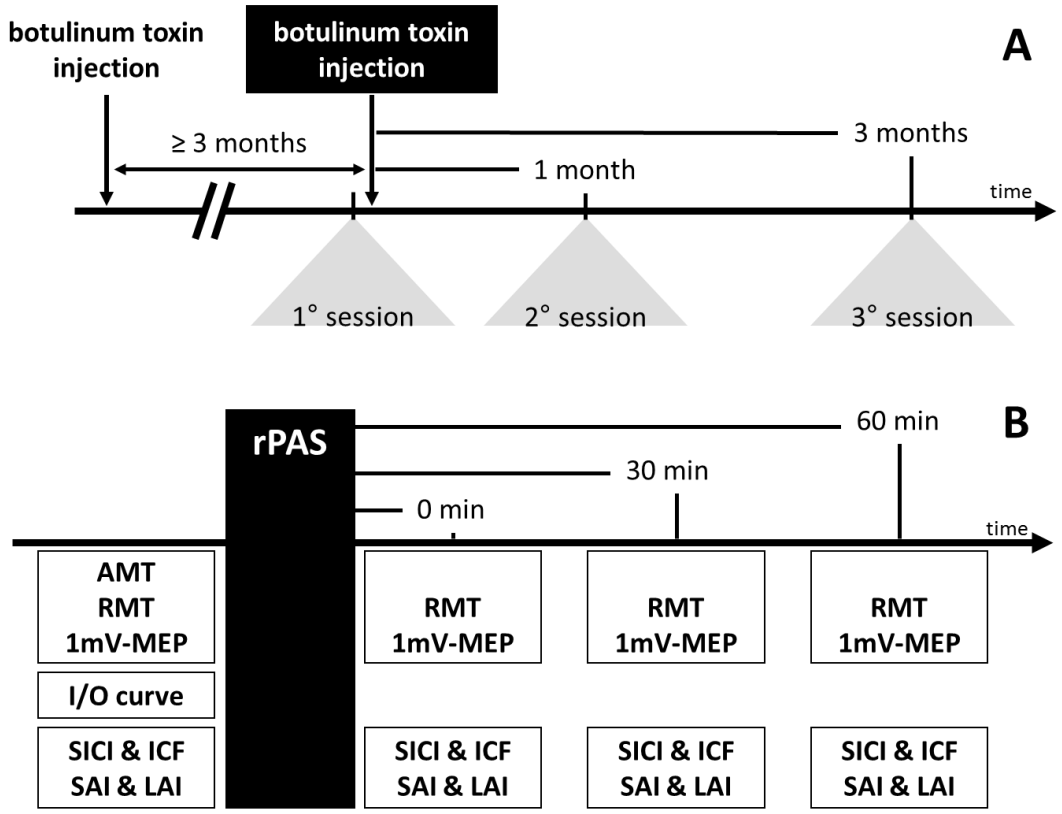


Figure 5.1 Experimental design

The experiment was designed (A) to study the response to rPAS before, 1 month and 3 months after BT injections. (B) In each session we measured: i) M1 excitability (AMT, RMT, 1mV-MEP and IO curve), ii) intra-cortical excitability (SICI and ICF) and iii) SAI and LAI. All measurements were repeated before rPAS, 0, 30 and 60 min after rPAS, except for the AMT and IO curve, which were measured only before rPAS.

5.3.4 Statistical Analysis

To test if the subjects with arm involvement had different responses to rPAS than the patients with isolated CD, we first used a preliminary 3- way ANOVA (ARM INVOLVEMENT x BT INJECTIONS x rPAS). Then, we used repeated measures ANOVA to determine the interaction between BT injections and our measures of interest: baseline measures of cortical excitability (including IO curves), response to rPAS, SICI, ICF, SAI and LAI. The clinical effect of BT injections was assessed by non-parametric Friedman's ANOVA. We also correlated the rPAS response at 30min (as a % of the pre rPAS MEP amplitude) in the first session with patients' demographic characteristics, using Spearman's correlation analysis. In addition, a correlation analysis was performed between normalised rPAS response at 30min (since the response peaked at 30 min) in the first, second and third session and the time elapsed since the previous BT injections (in weeks) and BFM score. Since in this analysis within subjects repeated measures are combined, the correlation coefficient was calculated according to the Bland and Altman correction (Bland and Altman, 1995).

5.4 Results

5.4.1 Clinical effect of BT injections

As expected, there was a clinical improvement of dystonia after BT injections (Friedman's χ^2 (2) =10.8, p<0.01). Post hoc analysis showed that the BFM score was significantly lower (indicating less severe dystonia) 1 month after injections than before and 3 months after BT injections (p< 0.01), while no significant difference was found in BFM score before and 3 months after BT injections.

5.4.2 BT injections into neck muscles do not modify baseline cortical-spinal excitability

BT did not change the RMT or AMT of the APB muscle. A two-way ANOVA comparing the MEP IO curve before rPAS in each of the three experimental sessions showed a significant effect of STIMULUS INTENSITY (F (2, 14) = 41.15; p<0.01) but no effect of BT INJECTIONS and no STIMULUS INTENSITY x BT INJECTIONS interaction **(Figure 5.2)**. Further analysis of the IO curve in the range of intensities from 90 to 110 % of 1mV MEP, which correspond to the range of MEP amplitudes before and after rPAS in all 3 experimental sessions, confirmed that there was a main effect of STIMULUS INTENSITY**,** but no effect of BT INJECTIONS or interaction INTENSITY X BT INJECTIONS.

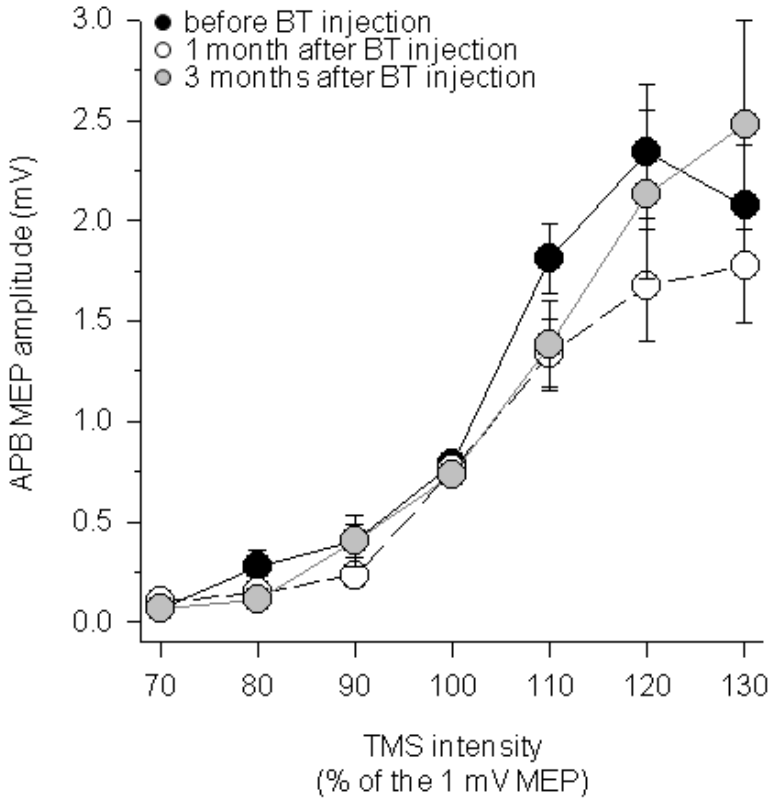


Figure 5.2 Effect of BT injections on the IO curve

There is no significant effect of BT injections on the IO curve.

5.4.3 BT injections reduce rPAS response in APB and FDI muscles

The results on the effect of BT injections on the response to rPAS are illustrated in **Figure 5.3**. Patients with additional arm involvement (6/12) did not differ from patients with isolated CD (6/12) in response to rPAS in any of the 3 experimental sessions. Therefore, all the subsequent analysis was done on the group of patients as a whole.

For the APB muscle, a two way ANOVA revealed a significant effect of BT INJECTIONS (3 levels : before BT, 1 month and 3 months after BT) ($F(2, 22) = 6.46$; $p<0.01$) and rPAS (4 levels: before rPAS, 0min, 30min and 60min after rPAS) ($F(3, 33) = 3.66$; $p<0.05$) as well as a significant interaction ($F(6, 66) = 3.06$; $p<0.01$)). Post hoc analysis showed for the factor BT INJECTIONS that the mean MEP amplitude was lower one month after BT injections comparing to the values before BT injections and 3 months after injections ($p<0.01$). Post hoc analysis for the main factor rPAS showed that the mean MEP amplitude was significantly higher 30 min after rPAS, comparing to the other time points ($p< 0.05$). The BT INJECTIONS X rPAS interaction was further explored by examining the main effect of rPAS separately within each experimental session. This showed a significant effect of rPAS before BT injections ($F(3, 33) = 4.86$, $p<0.01$) but not after 1 month ($F(3, 33) = 0.18$, $p>0.05$), nor after 3 months ($F(3, 33) = 1.85$, $p>0.05$). There was a non-significant trend for rPAS response to be greater at 3 months post BT compared to 1 month post BT.

In the FDI muscle ANOVA revealed a significant effect of BT INJECTIONS ($F(2.22) = 7.60$; $p<0.01$) while rPAS or interaction BT INJECTIONS X rPAS were non-significant. Similarly to the APB muscle, the mean FDI MEP amplitude after rPAS was lower one month after BT ($p<0.01$) compared to the values before BT, but no different from the value at 3 months**.**

To compare whether the APB and the FDI behaved similarly in response to rPAS and BT, (Quartarone et al., 2003, Quartarone et al., 2008) for each individual we expressed the average facilitation at 0, 30 and 60 min after rPAS as a percentage of the corresponding baseline values. A two way ANOVA with main factors MUSCLE (APB and FDI) and BT INJECTIONS revealed a significant effect of BT INJECTIONS ($F (2, 22) =4, 04$; $p<0.05$) but not of MUSCLE or interaction MUSCLE X BT INJECTIONS **.** We conclude that response to rPAS in relation to BT injections was similar in the APB and FDI muscles.

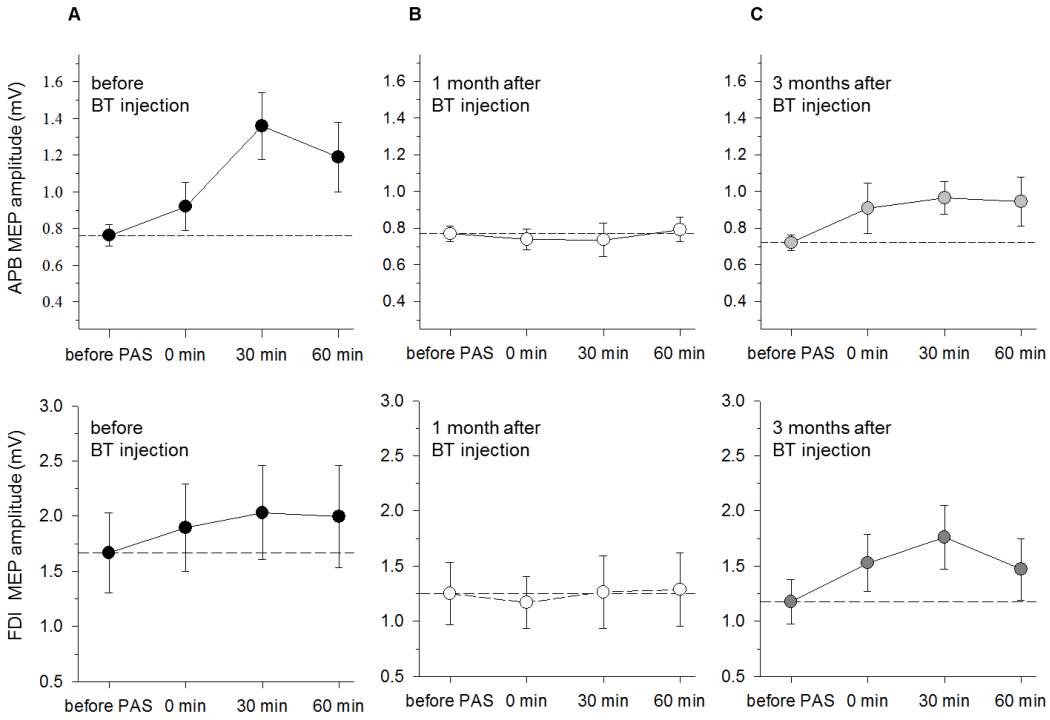


Figure 5.3 Effect of BT injections on the rPAS response

Botulinum toxin injections into dystonic neck muscles abolished the rPAS induced plasticity of the primary motor cortex hand area. Before BT injections (A) rPAS induced powerful plastic changes of hand cortical-spinal excitability: MEPs in APB and FDI muscles increased in amplitude immediately after rPAS (0 min), reaching a peak at 30 min. One month after BT injections (B) the rPAS response was completely abolished and it partially recovered three months later (C).

5.4.4 rPAS and BT do not modify intracortical excitability and sensory afferent inhibition from hand muscles

There was no effect of rPAS or BT injections on SICI and ICF.

There was no effect of rPAS or BT injections on SAI and LAI **(Figure 5.4)**.

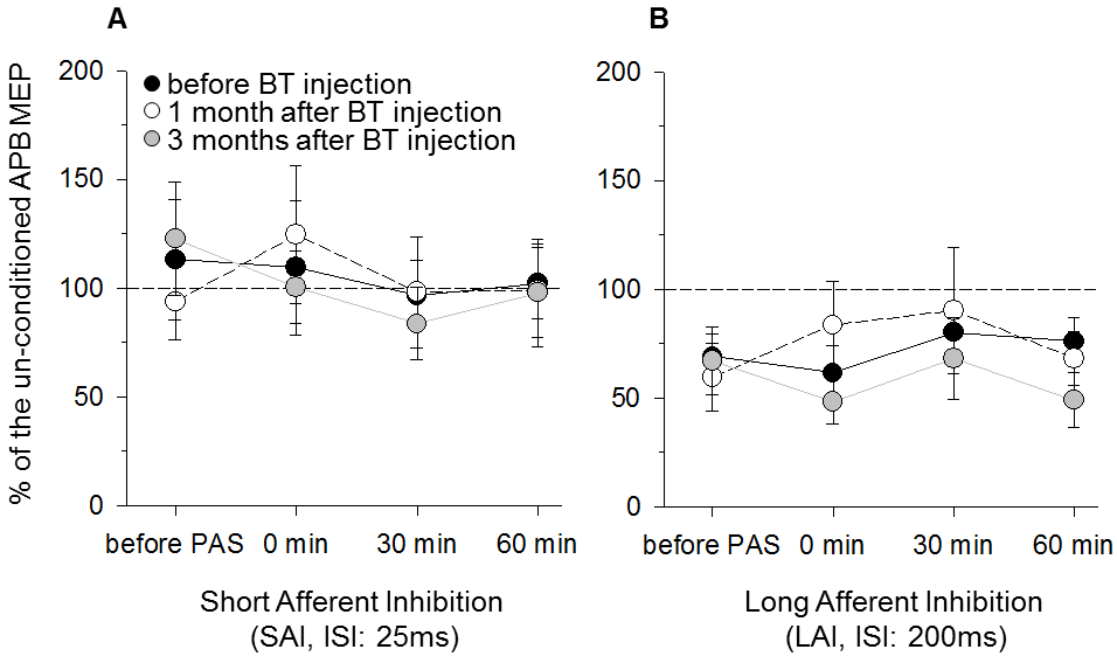


Figure 5.4 Effect of rPAS and BT injections on SAI and LAI

Patients with CD showed no short afferent inhibition (SAI) at an ISI of 25 ms (A), while long afferent inhibition (LAI) was present (B). Transmission in both SAI and LAI circuits was not modulated by BT injections or by rPAS.

5.4.5 rPAS induced plasticity correlates with dystonia severity and time after previous BT treatment

There was a significant positive correlation between the normalized MEP amplitude at 30 min after rPAS and (i) the time elapsed after BT injections ($R^2=0.37$, $p<0.01$) and (ii) the severity of dystonia assessed by BFM score ($R^2=0.30$ $p<0.01$) **(Figure 5.5)**. There were no significant correlations between patients' age, disease duration, duration of BT treatment or the dose of the last BT injections and the rPAS response.

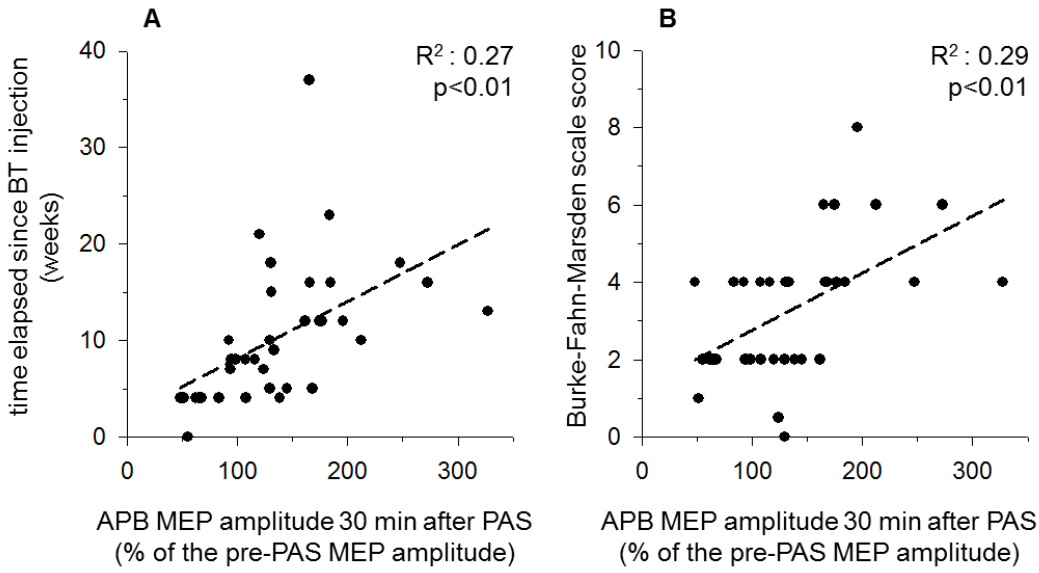


Figure 5.5 Correlation between rPAS induced plasticity of the primary motor cortex and timing of previous BT injection or clinical symptoms of dystonia.

Peak-to-peak amplitude of the normalised APB MEPs recorded 30 min after rPAS positively correlated with (A) the time elapsed since the previous BT injections in weeks and with (B) the Burke-Fahn-Marsden dystonia severity score. Each patient with their corresponding values of rPAS response and the number of weeks since the previous BT injections (A) or BFM score (B) is plotted 3 times -before BT injections, 1month and 3 months after BT injections.

5 5 Discussion

BT transiently abolished the response to an experimental sensorimotor plasticity protocol in patients with CD. Before BT injections, rPAS significantly facilitated MEP amplitude in the hand muscles. One month after injections this facilitation was suppressed, while at 3 months it partially recovered. The response to rPAS correlated significantly with the time elapsed after previous BT injections and with the clinical severity of dystonia as measured by BFM total score. We saw no clinical improvement in the severity of arm dystonia in those patients with CD with additional arm involvement, after injections into the neck muscles.

Possible BT action on the mechanism of cortical plasticity

BT can affect the release of neurotransmitters that are important for brain plasticity and is used in animal studies to block the connection between different brain areas, in order to study changes in brain plasticity (Ando et al., 2002, Costantin et al., 2005, Caleo et al., 2007) . Although there is some evidence to support hematogenous and axonal spread of large doses of BT in animals (Boroff and Chen, 1975, Antonucci et al., 2008) similar evidence in humans is lacking. Therefore a direct central effect of BT on plasticity in our patients is unlikely, particularly at the doses used for treatment of dystonia.

Another explanation for our results is that the effect of BT on cortical plasticity may be secondary to changes in the motor maps that occurred after afferent input from cervical muscles was altered by injections (Byrnes et al., 1998, Thickbroom et al., 2003). A considerable body of evidence demonstrates the importance of afferent input in modulating both cortical organization and its excitability (Brasil-Neto et al., 1992, Ridding and Rothwell, 1995, Ziemann et al., 1998a, Ziemann et al., 1998b). In dystonia, changes in "motor maps" of the hand muscles have been described after BT injections (Byrnes et al., 1998, Thickbroom et al., 2003). Thickbroom et al. (2003) reported that in patients with CD, motor maps of the APB are displaced in the hemisphere contralateral to the direction of head rotation. After BT injections into cervical muscles, the APB motor maps reverted to a more normal position, thus showing that changes in the motor cortical topography after injections may extend outside the area representing the treated muscles. This may be relevant for the experimental plasticity protocol we used. PAS relies on the interaction between sensory afferents and the motor output of the homologous muscle. If motor maps change in location after BT, then there may be a degree of disconnection between the sensory afferents and the altered location of the hand motor maps leading to a reduced PAS response as measured in the hand muscles.

Measures of sensorimotor cortical inhibition and their relation to BT injections and rPAS

LAI was present in our group of dystonia patients, and was not modified with rPAS or in relation to BT injections (**Figure 5.4 B**)**.** However, the SAI at an ISI of 25 ms was absent and was not modified after rPAS (**Figure 5.4 A**). We measured SAI at an ISI of 25ms based on previous data on healthy subjects from Quartarone et al. (2006a), who found that SAI at 25ms ISI is present and may be modulated by rPAS. When Kessler et al. (2005) studied SAI in patients with writer's cramp at ISIs ranging from 14 to 36 ms the strongest inhibition was present at an ISI of 20 ms. Therefore, it is possible that we may have missed SAI at shorter intervals. Alternatively, our findings of absent SAI in CD patients (while not the main objective of this study) might reflect an abnormality in afferent inhibition in CD.

Limitations of the study

We did not record the H reflex or F-waves to monitor possible changes in α motoneuron excitability secondary to BT. However, we did not find any effect of BT on RMT, AMT and IO curves, i.e. in parameters which test the excitability of the entire corticospinal tract including the α motoneuron. In line with our observations, Priori et al.(Priori et al., 1995) found that 1 month after BT injections to the arm, the Hmax:Mmax ratio is unchanged, thus suggesting that

BT does not affect α motoneuron excitability. We found that BT injections reduce cortical plasticity in chronically treated dystonia patients, but we cannot comment on whether a similar effect would be present in dystonia patients naïve to BT. Another limitation of the study is the lack of comparison with healthy controls. However, a number of previous studies on different forms of dystonia, including CD, have demonstrated that dystonia patients have abnormally enhanced responses to PAS protocols compared to normal subjects (Quartarone et al., 2003, Weise et al., 2006, Quartarone et al., 2008). Our primary interest was to assess the *change* in PAS response with BT injections rather than the absolute level of PAS response at baseline. In the context of this study we consider that a comparison group of CD patients treated with placebo injections or a healthy participant group given BT injections would not have been justified ethically.

Conclusion

BT injections into dystonic neck muscles decreased sensorimotor associative plasticity in the hand area in patients with CD. We propose that this central effect is mediated by changes in motor maps, caused by reduced afferent input from the neck muscles following injections. Modulation of sensorimotor plasticity by changing the afferent input from the dystonic muscles may perhaps contribute to the clinical benefit of BT injections in dystonia over and above the effects caused by weakness in the injected muscles.

Chapter 6 **Pathophysiological differences between secondary and primary dystonias: the roles of cortical plasticity and intracortical inhibition**

Primary dystonia is thought to be a disorder of the BG because the symptoms resemble those of patients who have anatomical lesions in the same regions of brain (secondary dystonia). However, these two groups of patients respond differently to therapy suggesting differences in the pathophysiological mechanisms. In the following experiments we investigated if primary and secondary dystonia share the same pattern of electrophysiological abnormalities. We used TMS to study cortical functional involvement and the eye-blink classical conditioning paradigm to assess cerebellar functional involvement.

The work presented in this Chapter is published in the form of a research article: Kojovic M, Pareés I, Kassavetis P, Palomar FJ, Mir P, Teo JT, Cordivari C, Rothwell JC, Bhatia KP and. Edwards MJ .Secondary and primary dystonia: pathophysiological differences. *Brain*. 2013 Jul;136(Pt 7):2038-49.

6.1 Summary

Pathophysiological deficits in primary dystonia are well characterised and include reduced inhibition at many levels of the motor system and increased plasticity, while emerging evidence suggests there are additional cerebellar deficits. We compared the electrophysiological features of primary and secondary dystonia, using TMS of the motor cortex and the eye blink classical conditioning (EBCC) paradigm, to test whether dystonic symptoms share the same underlying mechanism. 11 patients with hemidystonia caused by BG or thalamic lesions were tested over both hemispheres, corresponding to the clinically affected and non-affected side and were compared to ten patients with primary segmental dystonia with arm involvement and ten healthy participants of similar age. We measured RMT, AMT, IO curve, SICI and CSP. Plasticity was probed using PAS 25. In secondary dystonia cerebellar-dependent conditioning was measured using the EBCC paradigm and results were compared to the data obtained from primary dystonia patients previously in our lab using the same technique. We found no difference in the MTs, IO curves or CSP between the secondary and primary dystonia patients or the healthy controls. In secondary dystonia SICI was reduced on the affected side, while it was normal on the non -affected side. Secondary dystonia patients had a normal response to the plasticity protocol on both affected and non-affected side and a normal EBCC that was not different from healthy participants. In contrast, patients with primary dystonia showed increased cortical plasticity and

reduced EBCC. Normal motor cortex plasticity in secondary dystonia demonstrates that abnormally enhanced cortical plasticity is not required for clinical expression of dystonia, and the normal EBCC suggests an absence of functional cerebellar involvement in this form of dystonia. Reduced SICI on the side of the lesion may result from abnormal BG output or be a consequence of maintaining an abnormal dystonic posture. Dystonia appears to be a motor symptom that can reflect different pathophysiological states triggered by a variety of insults.

6.2 Introduction

Dystonia is a hyperkinetic movement disorder characterised by sustained muscle contraction leading to twisting, repetitive movements and abnormal postures of affected body parts (Fahn, 1988). In the absence of any pathological cause, Marsden et al. (1985) initially proposed that primary dystonia was a BG disease on the basis that the symptoms closely resembled those of some patients with identified lesions of the BG or their output pathways (now classified as secondary dystonia). The implication was that the similarity of symptoms was caused by a similar underlying pathophysiology. However, primary and secondary dystonias differ in their response to treatment (Neychev et al., 2011); in addition there is emerging evidence that a cerebellar deficit may contribute to the symptoms of primary dystonia (Sadnicka et al., 2012) .Given the etiological and clinical heterogeneity of dystonia, the aim of the present

study was to test whether the primary and secondary forms share a similar pathophysiological mechanism.

Most electrophysiological and neuroimaging studies in dystonia have been conducted on patients with primary dystonia since this is the commonest form of the condition (Bressman, 2004). A consistent finding is loss of inhibition at different levels of the CNS, including spinal cord, brainstem and motor cortex (Berardelli et al., 1985, Nakashima et al., 1989, Ridding et al., 1995b). Recent evidence from human studies suggests that abnormally enhanced synaptic plasticity is also an important factor in the pathophysiology of the primary dystonias (Peterson et al., 2010, Quartarone and Pisani, 2011). Patients with primary focal and primary generalised dystonia have an abnormally enhanced response to different plasticity protocols that probe LTP-like and LTD-like synaptic plasticity in the motor cortex (Quartarone et al., 2003, Edwards et al., 2006, Weise et al., 2006, Gilio et al., 2007, Quartarone et al., 2008) or brainstem circuits (Quartarone et al., 2006b). Finally, a range of recent evidence from structural and functional imaging and electrophysiology (Teo et al., 2009) suggests that the cerebellum may also play some role in primary dystonia. Thus, voxel based morphometric studies have found grey matter changes in the cerebellum of patients with focal dystonias (Draganski et al., 2003, Delmaire et al., 2007, Obermann et al., 2007), functional MRI has revealed changes in movement-related activity (Odergren et al., 1998, Carbon and Eidelberg, 2009) and metabolic profile (Hutchinson et al., 2000).

Although there are some reports that patients with secondary dystonia may share similar abnormalities in inhibitory networks of the motor system (Nakashima et al., 1989, Trompetto et al., 2012), there is no information about plasticity or cerebellar function in this group of patients. The aim of the present study was to provide a more comprehensive comparison of the underlying pathophysiology in primary and secondary dystonias. The results show that there are distinct differences in physiology, implying that the clinical syndrome of dystonia has more than one physiological phenotype. This would be consistent with the fact that dystonia can have many different causes and can respond quite differently to treatment (Neychev et al., 2011). The conclusion is that dystonia represents one (of many) possible stable state(s) into which the motor system can be pushed through a variety of insults.

6.3 Methods

6.3.1 Participants

We studied 11 patients with secondary dystonia caused by a structural brain lesion (5 men and 6 women, mean age 45.8 years, range 28-68, **Table 6.1**), 10 patients with primary segmental dystonia (4 men and 6 women, mean age 46.7 years, range 31-67, **Table 6.2**) and 10 age -matched healthy participants (5 men and 5 women, mean age 48.7 years, range 27-67). Patients with secondary dystonia were included if they had: (i) unilateral distribution of

dystonia (ii)a discrete lesion in the BG and/or thalamus contralateral to the clinically involved side on magnetic resonance imaging or computed tomography and (iii) no significant pyramidal involvement or hemisensory loss, as assessed by the Ashworth scale and NIH Stroke Scale. All patients were clinically examined and videotaped. Three patients with secondary dystonia had resting dystonia with fixed postures (patient 1 and 2 fixed dystonia of the leg, patient 5 fixed dystonia of the arm), the other eight patients had mobile dystonia at rest, worsened by action. All patients with primary dystonia had segmental dystonia with unilateral arm involvement visible at rest or on maintaining an outstretched arm. Clinical disease severity was assessed with Burke-Fahn-Marsden (BFM) scale. All patients treated with BT were injected at least 15 weeks before participating in the study. One of the secondary dystonia patients (patient 5) had undergone unilateral thalamotomy 20 years earlier, with only transient improvement of symptoms. At the time of the study, none of the participants were on any medications that we believe could affect the measurements performed. All the participants were right-handed. EBCC testing was performed on patients with secondary dystonia and, for convenience their data on EBCC were compared to the data of primary dystonia patients (7 males, 6 fames, mean age 63.7 +/- 3.4 (SEM)) and healthy participants (6 males, 5 females, mean age 61 +/- 4.5 (SEM)) obtained in our laboratory using the same experimental protocol (Teo et al.,2009).

Table 6.1 Clinical and demographic characteristic of secondary dystonia patients

Patient	Age(yrs) /Gender	Dystonia onset (yrs)	Disease duration (yrs)	Distribution of dystonia and characteristics	Cause	Lesion on MRI	BFM score	NIHSS score	Duration of BT treatment (yrs)
1	38/F	28	10	L hemidystonia; fixed at foot, mobile at arm	Ischemic stroke	R pallidum	9	0	2 (Discontinued In last 4 yrs)
2	63/F	32	29	L hemidystonia; fixed at foot, mobile at arm	Ischemic stroke	R striatum	21	2	Not treated
3	40/M	2	38	R hemidystonia; mobile	Perinatal HII	L thalamus	28	1	5
4	68/M	55	13	R hemidystonia; mobile	Ischemic stroke	L thalamus	18	1	Not treated
5	63/F	2	61	L hemidystonia; fixed at arm, mobile at leg	Perinatal HII	R lent.nc.	27	2	15
6	38/M	6	32	L hemidystonia; mobile	Ischemic stroke	R lent.nc.	16	2	Not treated
7	28/M	2	26	L arm; mobile	Perinatal HII	R lent.nc.	19	3	6
8	36/F	3	33	L hemidystonia; mobile	Encephalitis	R lent. nc.	27.5	1	7
9	48/F	1	47	L hemidystonia; mobile	Perinatal HII	R lent. nc.	21.5	0	18
10	42/M	18	24	R hemidystonia; mobile	Ischemic stroke	L striatum	26	2	10
11	40/F	1	39	R hemidystonia; mobile	Perinatal HII	L lent. nc.	13	0	Not treated
Average +/- SE	**45.8 +/-13.8**	**13.6 +/-4.1**	**32 +/-9.6**				**20.5 +/-6.2**	**1.3 +/-0.4**	**5.7 +/-1.7**

Table 6.2 Clinical and demographic characteristic of primary dystonia patients

Patient	Age(yrs) /Gender	Age of dystonia onset	Disease duration(yrs)	Distribution of dystonia and characteristics	BFM score	Duration of BT treatment (yrs)
1	39/F	29	10	CD and R arm dystonia	12	5
2	63/F	59	4	BSP, CD and R arm dystonia	13	2
3	29/F	23	6	CD and R arm dystonia	9	Not treated
4	44/M	40	4	CD and L arm dystonia	8	2
5	31/M	24	7	CD and R hand dystonia	8	Not treated
6	53/F	47	6	Laryngeal dystonia, CD and L arm dystonia	26	6
7	40/F	32	8	CD and R arm dystonia	12	2
8	67/M	20	47	Laryngeal dystonia and L arm dystonia, L dystonic tremor	9	26
9	50/M	43	7	CD and R hand dystonia	16	5
10	51/M	29	10	BSP, CD and R arm dystonia	9	5
Average +/- SE	**46.7 +/-14.8**	**34.6 +/-10.9**	**10.9 +/-3.4**		**12.2 +/-39**	**5.3 +/-1.7**

Abbreviations: yrs: years; BFM: Burke-Fahn-Marsden dystonia score; BT: Botulinum Toxin; F: Female; M: Male; BSP, blepharospasm; CD, cervical dystonia; R: Right; L: Left

6.3.2 EMG recordings and TMS

EMG recordings were made from the APB and ADM on the side contralateral to the stimulated hemisphere. We used single-pulse and paired-pulse TMS and PAS25 protocol, as indicated in Chapter 2. SICI was assessed at an ISI of 2 ms.

6.3.3 Eye blink classical conditioning (EBCC)

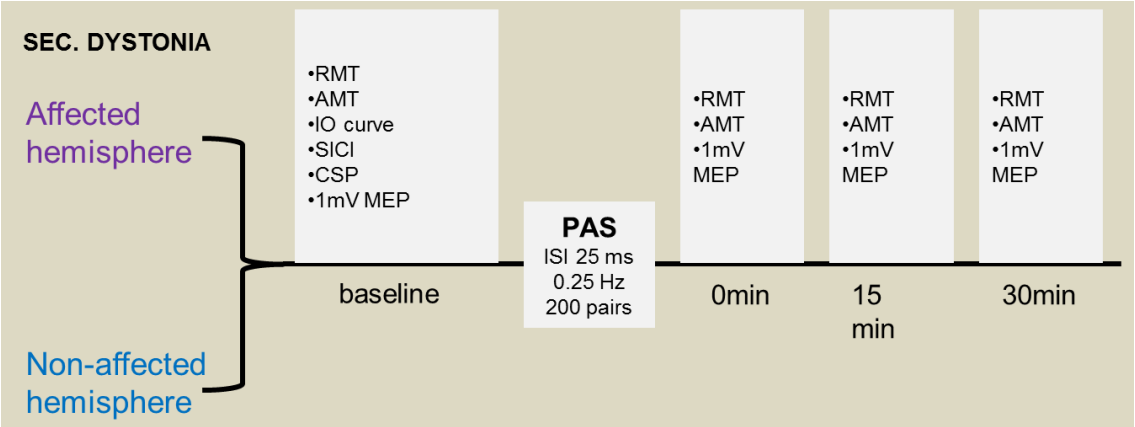
An electrical stimulus was applied through a bipolar electrode, with the cathode positioned proximally. The electrical stimuli were constant current square wave pulses with a pulse width of 200 μs, i.e. unconditioining stimulus (US) and were delivered to the right supraorbital nerve at an intensity adjusted to obtain stable R2 responses (approximately 4-6 times the sensory threshold). Electrical supraorbital nerve stimulus was preceded by a tone, i.e. the conditioning stimulus (CS), produced by a tone generator and presented bilaterally to the subject via binaural headphones at an intensity 50–70 dB above the individual hearing threshold. The CS intensity was kept constant during experiment. The CS inconsistently produced an acoustic startle response (alpha blink) occurring within 200 ms after the CS onset. Repeated pairs of CS and US at 400 ms intervals induced a conditioned eye blink response (CR) to appear with onsets within 200 ms before US. EBCC sessions consisted of seven blocks: six acquisition blocks (each block contained 11 trials in: nine trials of CS–US pairs,

the 10th trial was US only and trial 11th was CS only) followed by one extinction block (11 trials of CS only). For measurement of EBCC, the CRs were counted manually. EMG bursts were regarded as ‘‘alpha blinks’’ if their amplitude exceeded 50 μV and if latency was < 200 ms after the CS. EMG bursts were regarded as CRs if latency was > 200 ms after the CS but before the US . For the CS only trials, EMG bursts occurring 200–600 ms after the CS were considered CR.

6.3.4 Experimental design

Secondary dystonia patients were tested on both hemispheres, corresponding to the clinically affected and non-affected side in two different TMS sessions, separated by at least one week (**Figure 6.1**). The order of the tested hemisphere (affected vs. unaffected) was balanced between subjects. Primary dystonia patients were tested on the hemisphere corresponding to the affected side only, since previous studies had showed that in primary dystonia abnormalities in TMS measures are present in the affected and unaffected parts of the body (Quartarone et al., 2008). Healthy participants were tested on the dominant hemisphere only. In each session we began with baseline assessments of RMT, AMT and 1mV- MEP, IO curve, SICI and CSP. We then delivered PAS25 and assessed the effect of this conditioning protocol on corticopinal excitability (1mV- MEPs) at three different time points: 0, 15 min

and 30 min after PAS. In addition, secondary dystonia patients underwent a third session for EBCC testing.



- Primary dystonia- Affected side
- Healthy controls- Dominant hemisphere

Figure 6.1 Experimental design

6.3.5 Statistical analysis

ANOVA was used to test the age differences between secondary dystonia patients, primary dystonia patients and healthy controls. The differences in disease duration, BFM scores and duration of BT treatment between secondary and primary dystonia patients were assessed with Mann-Whitney U test.

The primary aim of this study was to compare the TMS parameters between the affected side in secondary dystonia patients and primary dystonia patients and healthy participants. RMT, AMT, EMG root square amplitude, SICI and CSP were compared between groups using ANOVAs with a factor GROUP (3 levels: secondary dystonia-affected side, primary dystonia and healthy participants) as a between-subject factor. For analysis of SICI, conditioned MEP amplitudes were averaged, normalized to average unconditioned MEP amplitudes and entered into ANOVA with factor GROUP as between-subjects factor. IO curves were compared between groups in ANOVA with the factor GROUP and the factor STIMULUS INTENSITY (10 levels of stimulator output intensity ranging from 80 % to 170% of RMT intensity) as a within-subjects factor. For analysis of the PAS effect, MEP amplitudes at each time point were averaged, normalized to baseline MEPs and entered into ANOVA with factor GROUP as between-subjects factor and factors MUSCLE (2 levels: APB and ADM muscle) and TIME POINT (3 levels: 0 min, 15 min and 30 min after PAS) as a within-subjects factors. As a secondary analysis, we assessed how TMS measures

compared between the affected and non-affected side in secondary dystonia patients, using repeated measures ANOVA or paired sample t-test. For EBCC, the percentage of CRs over different blocks did not follow the normal distribution, therefore non-parametric tests were used. We first compared the number of overall CR (for all blocks) in each group using Kruskal -Wallis ANOVA. The differences in the number of CR in each block between groups were then assessed by Mann-Whitney U test. Finally, for each group we used Friedman ANOVA to test if there was a conditioning of eye blink responses across blocks.

Possible correlations between clinical and demographic data (disease duration, BFM score, duration of BT injection treatment) and TMS measures (SICI, averaged PAS response) were evaluated with the Spearman correlation analysis.

6.4 Results

6.4.1 Clinical and demographical data

There was no significant difference in age between the secondary and primary dystonia patients and healthy participants. As expected, the BFM score was higher in the secondary compared to primary dystonia patients (z=-2.9;

p=0.004) and also the disease duration was longer in the secondary dystonia patients (z= -3.14; p=0.002). No difference was found in the duration of BT treatments between the dystonia groups (z=-0.72; p=0.93).

6.4.2 Corticospinal excitability and EMG root mean square amplitude

At baseline, no significant difference was found in RMT, AMT, 1mV MEPs TMS intensity or EMG root mean square amplitude between secondary dystonia and primary dystonia patients and healthy participants or between affected and non-affected sides in secondary dystonia patients.

As expected, for IO curves ANOVA showed a significant effect of STIMULUS INTENSITY ($F(9, 207) = 28.9$; $p < 10^{-3}$), due to an increase of MEP size with increasing TMS intensity, while the factor GROUP and the interaction GROUP X STIMULUS INTENSITY were both non-significant. The side comparison in secondary dystonia, also revealed a significant effect of STIMULUS INTENSITY ($F(9, 36) = 13.6$; $p < 10^{-3}$while the main factor SIDE and the interaction SIDE X STIMULUS INTENSITY were both non- significant. These results overall indicate that there was no difference in baseline corticopinal excitability between secondary dystonia and primary dystonia patients and healthy participants (**Figure 6.2**) or between affected and non-affected sides in secondary dystonia patients (**Figure 6.3**).

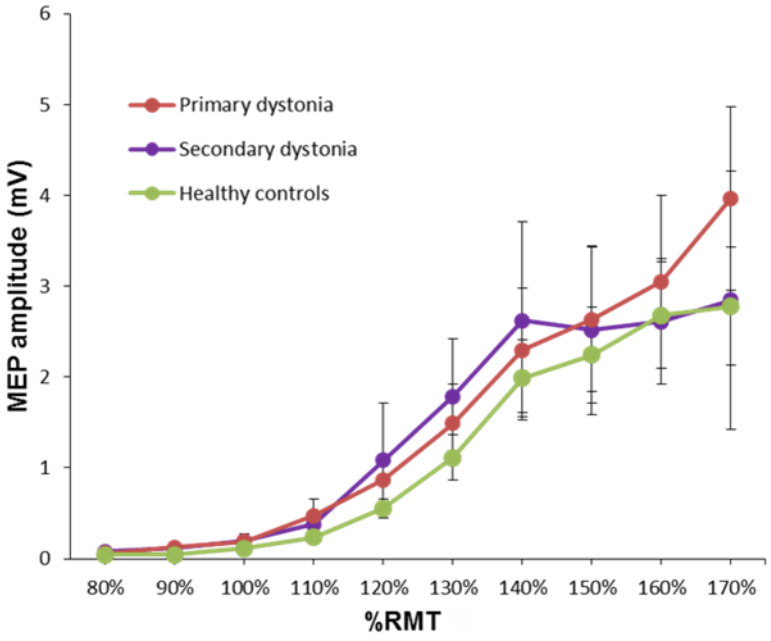


Figure 6.2 IO curves

The IO curves in secondary dystonia patients, primary dystonia patients and healthy participants are not significantly different.

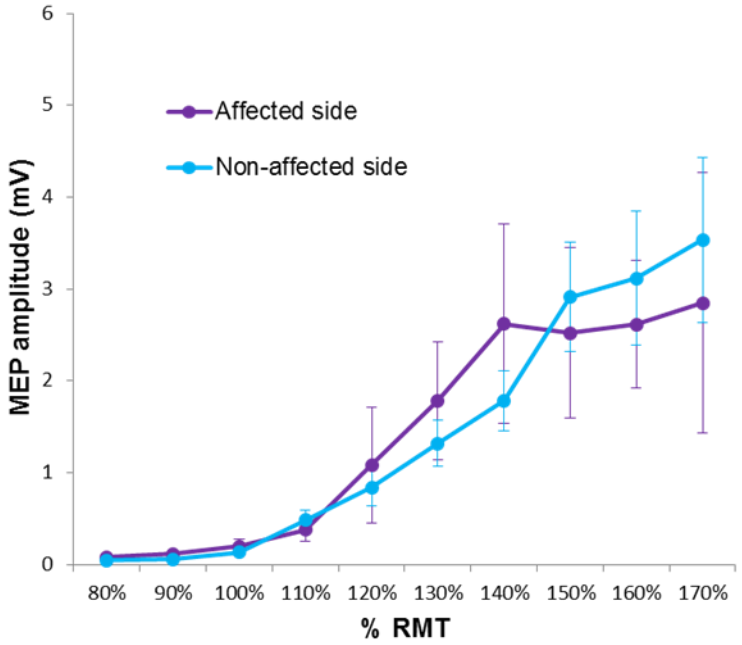


Figure 6.3 IO curves in affected and non-affected side in secondary dystonia patients

There is no difference in the IO curves between the affected and non-affected sides of secondary dystonia patients.

6.4.3 SICI

ANOVA revealed a significant effect of factor GROUP ($F(2, 27) = 5.11$; $p = 0.01$), due to less SICI in secondary dystonia patients compared to healthy participants ($p=0.01$), while there was no difference between primary and secondary dystonia or between primary dystonia and healthy participants. When the affected side was compared to the non-affected side in secondary dystonia, a paired-sample t-test revealed significant difference ($p=0.02$) due to less SICI on the more affected side (**Figure 6.4**).

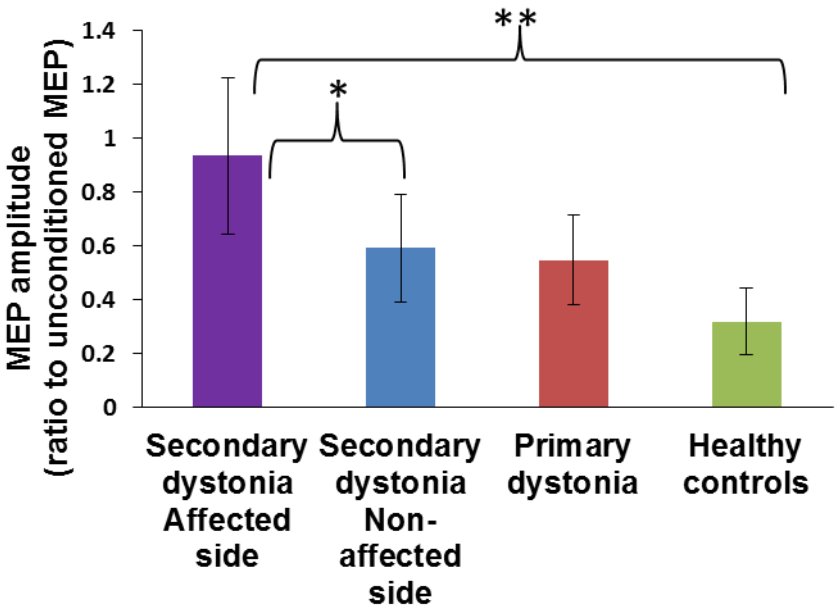


Figure 6.4 Short-interval intracortical inhibition

In secondary dystonia patients, SICI is reduced on the affected side, compared to the non-affected side and to healthy participants. Data is plotted as a ratio to the unconditioned MEP amplitude. (** p ≤0.01; *p<0.05)

6.4.4 CSP

ANOVA revealed no difference in CSP between secondary dystonia and primary dystonia patients and healthy participants. Also paired-sample t-test revealed no difference in CSP between affected and non-affected sides in secondary dystonia (**Figure 6.5**).

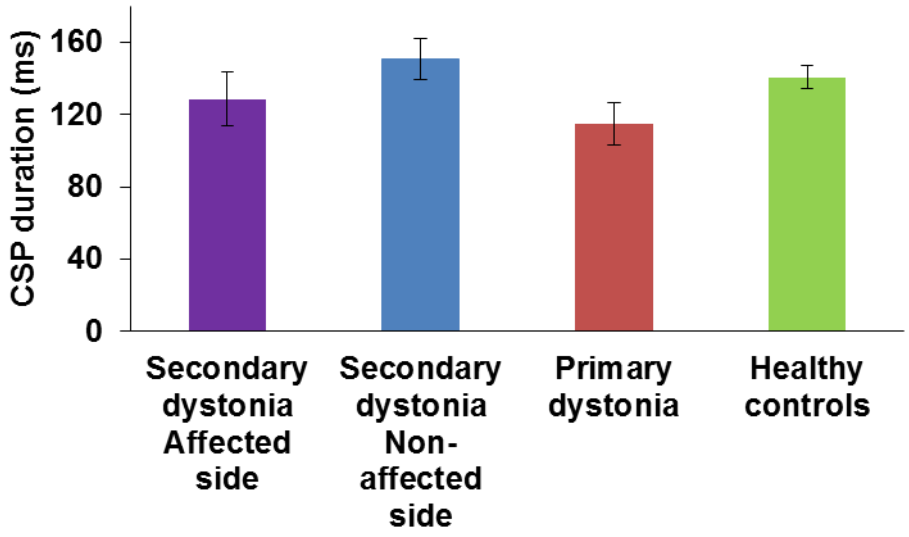


Figure 6.5 Cortical Silent Period

There is no difference in the CSP duration between secondary dystonia patients, primary dystonia patients and healthy participants.

6.4.5 PAS

There was no within-or between-subject difference in electrical stimuli counting errors during PAS, thus excluding differences in attention levels in different sessions.

The results of PAS effect are given in **Figures 6.6, 6.7** and **6.8**. **Figure 6.9** represents averaged PAS response in individual participants. ANOVA revealed significant effect of the factor GROUP ($F(2, 28) = 12$; $p < 10^{-3}$), due to higher response to PAS in primary dystonia patients comparing to both secondary dystonia patients ($p < 10^{-3)}$ and healthy participants ($p < 10^{-3}$), while there was no difference between secondary dystonia patients and healthy participants. Factors MUSCLE and TIME POINT were not significant and all 2 -way and 3-way interactions were also not significant, indicating that PAS response was higher in primary dystonia at all 3 time points after the PAS and in both APB and ADM muscles (**Figure 6.6 and 6.7**). When the affected side was compared to non-affected side in secondary dystonia, ANOVA revealed the significant effect of the factor MUSCLE ($F(1, 9) = 8.7$; $p=0.02$), due to higher response to PAS in the APB compared to the ADM muscle. Factors SIDE and TIME POINT were not significant as were the interactions between main factors, indicating that there was no difference in the PAS response between the affected and

non-affected side in secondary dystonia and that there was no spread of the PAS effect to the ADM muscle on either side (**Figure 6.8**.).

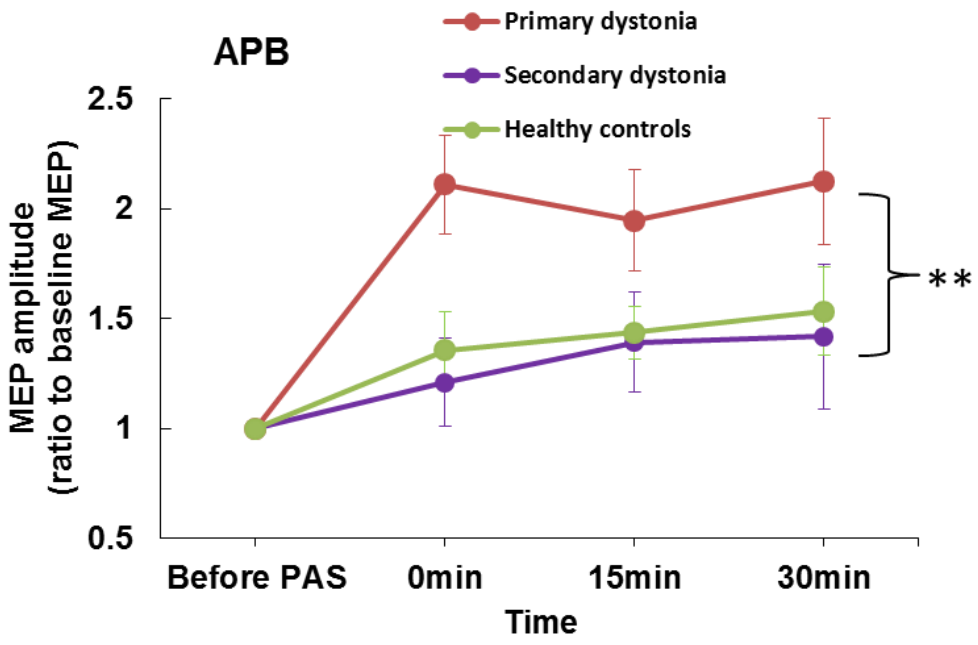


Figure 6.6 PAS effect on corticospinal excitability, as measured by change in 1mV MEP amplitude in APB

In the APB muscle, primary dystonia patients have a higher response to PAS at all 3 time points (i.e. 0 min, 15 min and 30 min after PAS) compared to secondary dystonia patients and healthy participants(** p ≤0.01). There is no difference in PAS response between the secondary dystonia patients and healthy participants.

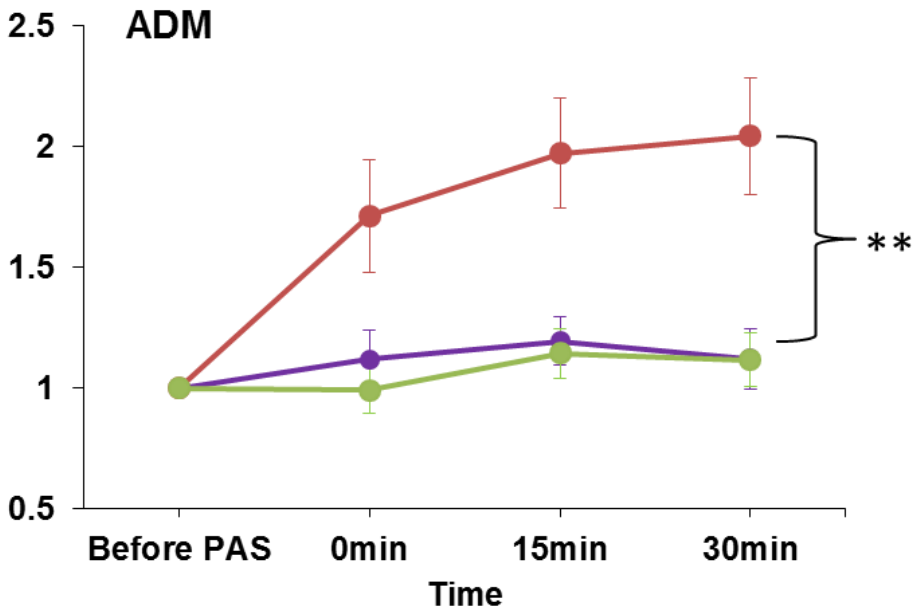


Figure 6.7 PAS effect on corticospinal excitability, as measured by change in 1mV MEP amplitude in ADM

Primary dystonia patients have a spread of PAS effect in the non-median innervated ADM muscle (** p ≤0.01), that is not present in secondary dystonia patients or healthy participants.

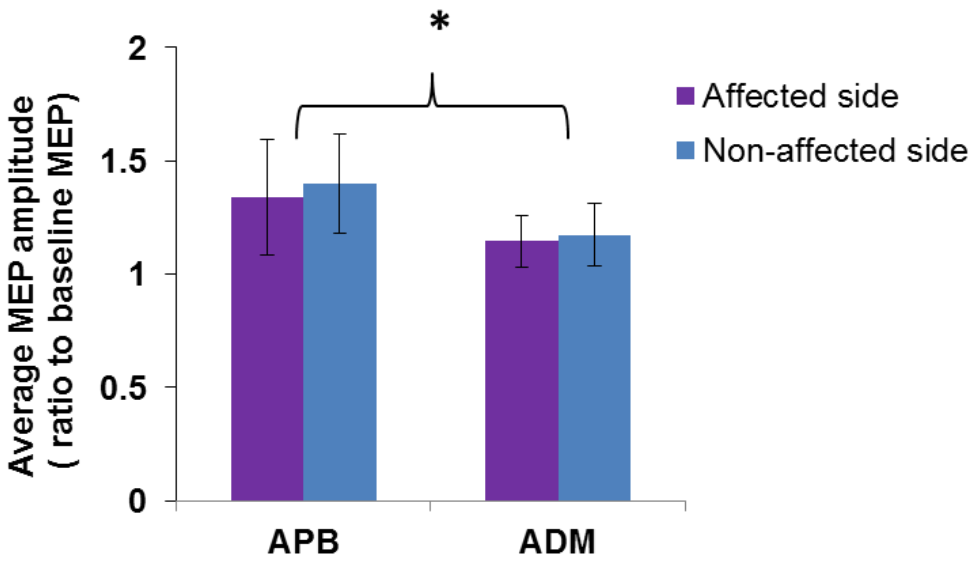


Figure 6.8 PAS response on affected and non-affected side in secondary dystonia patients

There is no difference in the PAS response between the affected and non-affected side in secondary dystonia patients. On both the affected and non-affected side, the PAS response is larger in the APB compared to ADM muscle (* $p \leq 0.05$).

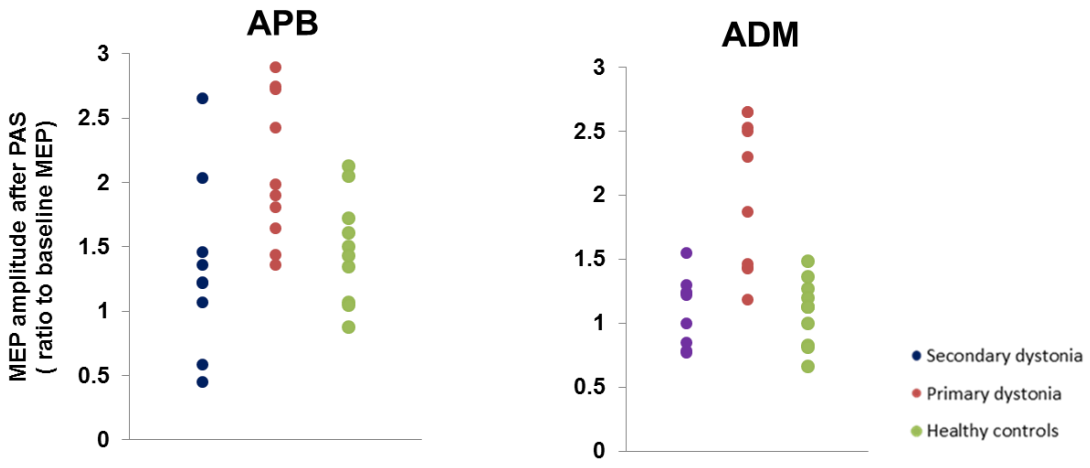


Figure 6.9 Averaged PAS response in individual participants.

For each participant, the PAS response is expressed as an averaged MEP amplitude for 3 time points after PAS (0 min, 15 min and 30 min after PAS) and is plotted on y-axis. For secondary dystonia the data refer to the affected side.

Table 6.3 Statistics of Eye-blink Classical Conditioning (Mann-Whitney U tests)

	Block 1	**Block 2**	**Block 3**	**Block 4**	**Block 5**	**Block 6**
Secondary Dystonia vs. Primary Dystonia	Z=-0.90 p=0.4	Z=-2.05 p=0,04	Z=-2.54 P=0.01	Z=-3.19 P=0.001	Z=-2.93 P=0.003	Z=-.62 P=0.009
Secondary Dystonia vs. Healthy Participants	Z=-0.74 P=0.5	Z=-1.77 P=0.08	Z=-1.15 P=0.2	Z-=0.76 P=0.5	Z=-0.36 P=0.7	Z=-0.96 P=0.3
Primary Dystonia vs. Healthy Participants	Z=-1.71 P=0.09	Z=-0.27 P=0.8	Z=-1.18 P=0.2	Z=-2.49 P=0.01	Z=-2.74 P=0.006	Z=-2.41 P=0.02

6.4.6 EBCC

ANOVA revealed significant difference in the ages of the compared groups ($F(2, 32) = 5.9$; $p = 0.006$), because our secondary dystonia patients were younger than the historical primary dystonia controls ($p=0.007$) and healthy participants ($p=0.03$).

Kruskal-Wallis ANOVA revealed a significant effect of the factor GROUP (χ^2 (2, N = 34) = 10.2; $p=0.006$). Post-hoc Mann-Whitney U tests showed that that this was due to more CR in the secondary compared to the primary dystonia in block 2-6 and more CR in healthy participants compared to primary dystonia patients (blocks 3-6) (**Table 6.3**). There was however no difference between the secondary dystonia patients and healthy participants. We further confirmed with Friedman ANOVA that the number of CR increased over blocks in both secondary dystonia patients ($\chi^2= 22.4$; $p<10^{-3}$) and healthy participants ($\chi^2= 22.9$; $p<10^{-3}$), but not in the primary dystonia patients ($\chi^2= 3.53$; $p=0.6$) (**Figure 6.10**).

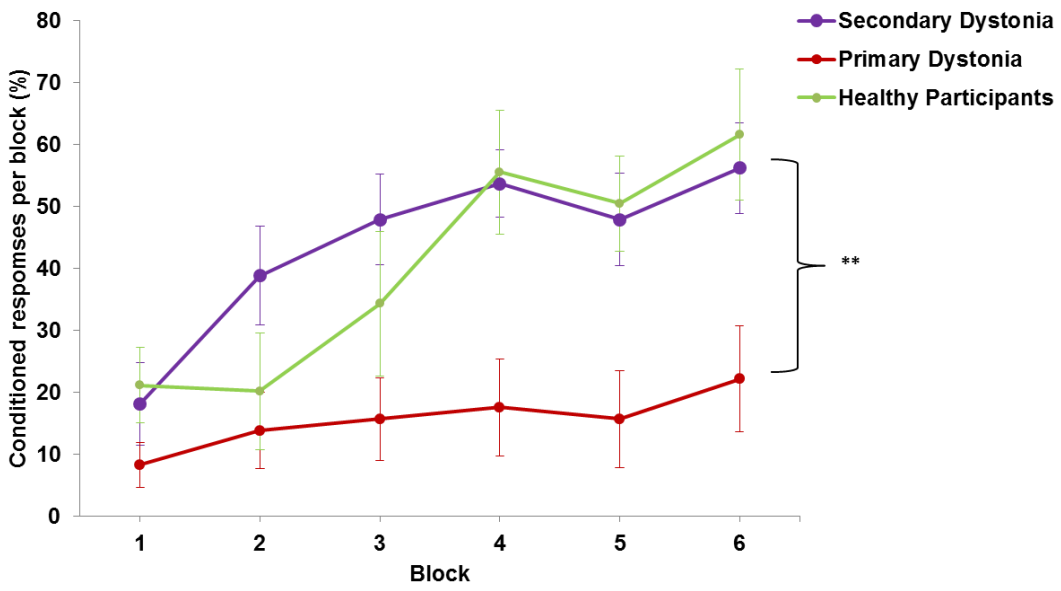


Figure 6.10 Eye Blink Classical Conditioning (EBCC)

Patients with secondary dystonia have significantly more conditioned eye blink responses compared to primary dystonia patients. Note that the secondary dystonia patient' data in the present study are compared to historical data from primary dystonia patients and healthy participants obtained in our laboratory using the same experimental protocol.

We found no significant correlation between the clinical and demographic data and TMS measures in our patients.

6.5 Discussion

The main findings of the present study are: (i) in secondary dystonia patients a response to PAS is no different than in healthy participants, in contrast to the enhanced response of patients with primary dystonia; (ii) secondary dystonia patients have reduced SICI, on the side of the lesion only; and (iii) EBCC is worse in patients with primary than in those with secondary dystonia.

Differences in PAS induced plasticity between secondary and primary dystonia

The enhanced response to PAS that we found in our patients with primary dystonia is in line with many previous studies using a variety of plasticity-testing protocols (Quartarone et al., 2003, Edwards et al., 2006, Weise et al., 2006). It was, however, surprising to find that the response to PAS was normal in secondary dystonia. This is unlikely to be due to differences in baseline corticospinal excitability, as the IO curves and motor thresholds were the same in all three groups that we studied. Nor is it likely to be a result of the longer duration and more severe dystonic symptoms in the patients with secondary dystonia. Although the present study only examined cases of primary segmental

dystonia, previous investigations from this laboratory have found enhanced responses to experimental plasticity protocols even in patients with primary generalised dystonia, whose symptoms began in childhood (Edwards et al, 2006) and were so severe as to require bilateral pallidal deep brain stimulation (Ruge et al, 2011). In addition, there was no correlation between disease duration and the response to PAS in our secondary dystonia patients.

As we found in the study described in Chapter 5 , BT treatment can transiently reduce the response to PAS in patients with primary dystonia, which then returns to levels found before BT injection after a few months. Since all the present patients were studied at least 15 weeks after their last injection, this acute effect of BT is unlikely to have influenced the present results. Nevertheless, it is difficult to speculate on whether there might have been possible chronic effects of BT on motor cortex plasticity, as this has not been previously investigated. Several of the patients with secondary dystonia had been treated for many years and it is possible that this could have permanently reduced their PAS response and skewed the group data even though there was no difference in the mean duration of treatment in the primary and secondary cases. This seems unlikely to have been the case as there was no correlation between the duration of BT treatment and the response to PAS protocol.

In the absence of other explanations, we suggest that enhanced motor cortex plasticity is an inherent, genetically determined trait (endophenotype) specific

for primary dystonia that predisposes some individuals to develop dystonia. As suggested by Quartarone et al .(2006c) this may result in an excessive tendency to form associations between sensory input and motor output, leading to dystonia, particularly under circumstances involving frequent repetition of specific movements . In contrast, secondary dystonia is believed to be related to functional changes in sensorimotor circuits following brain injury (Burke et al., 1980). The exact mechanism underlying the changes resulting in secondary dystonia and the anatomical regions in which they occur are not well understood but it may be that the principal pathological processes spare the function of the primary motor cortex. Thus, using PET activation study Ceballos-Baumann et al. (1995) showed that pattern of primary motor cortex activity differs between acquired hemidystonia and idiopathic torsion dystonia patients. Similarly, a combined fMRI and DTI study on a patient with hemidystonia caused by penetrating injury of caudate and lentiform nucleus showed that there was no significant functional reorganisation in the primary motor cortex after injury (Werring et al., 1998). This would be consistent with our finding of a normal response to PAS in our patients.

EBCC and its possible relation to PAS response in dystonia

EBCC, as used in human studies, is a form of predictive learning that lesion studies have shown to depend on the integrity of the olivo-cerebellar circuit (Gerwig et al., 2007). Indeed, in healthy individuals, continuous theta burst

stimulation (cTBS) over cerebellum, which is thought to interfere with function in cerebellar circuits, abolishes EBCC (Hoffland et al., 2012). Previously we had found that EBCC was markedly reduced compared to healthy volunteers in patients with primary focal hand and/or cervical dystonia and had speculated that this was further evidence in favour of cerebellar involvement in primary dystonias (Teo et al., 2009). In the present study, our patients with secondary dystonia showed preserved EBCC that did not differ from that of healthy controls. EBCC decreases with age, (Finkbiner and Woodruff-Pak, 1991, Bellebaum and Daum, 2004) and therefore the age difference between the different groups could have been a confounding factor. However, even though our secondary dystonia patients were younger than both healthy controls and primary dystonia patients, their EBCC was similar to that of healthy controls and superior to EBCC in primary dystonia. Therefore, a younger age is unlikely to be a reason for the apparently normal EBCC in our secondary dystonia patients. The implication of our findings is that the pathophysiology of secondary dystonia is more localised than that of primary dystonia.

Although EBCC and PAS are usually thought to test quite different circuits in different parts of the brain, there may be some connection between them that could potentially link the present results in primary and secondary dystonias. Recent work has shown that the response to some PAS protocols is modulated by inputs from the cerebellum; thus a disordered cerebellum could potentially lead to abnormal PAS. In healthy volunteers, the effect of a PAS25 protocol

(that is, with an interval of 25ms between median nerve and TMS pulse) is reduced or abolished by concurrent anodal direct current stimulation over the cerebellum or by preconditioning with excitatory intermittent theta burst stimulation (Hamada et al., 2012a); in contrast, preconditioning the cerebellum with cTBS enhanced PAS (Popa et al., 2012). Thus, the effect of motor cortex PAS25 depends on the functional state of the cerebellar output.

From the data outlined above, the combination of enhanced response to PAS25 and decreased EBCC in primary dystonia is similar to what occurs with cerebellar cTBS in healthy volunteers: EBCC is reduced and PAS25 plasticity increased. The conclusion is that a cerebellar disorder in patients with primary dystonia could contribute to their abnormal response to PAS. However, this is unlikely to be the whole story. The response to PAS21.5 (that is, PAS with a 21.5ms interval between stimuli) is unaffected by cerebellar direct current stimulation (Hamada et al., 2012a) in healthy participants yet it is still enhanced in primary dystonias (Weise et al., 2006), suggesting that there is an intrinsic disorder of cortical plasticity in addition to any secondary influence from a disordered cerebellum.

The role of reduced intracortical inhibition in dystonia

The final finding of our study is that secondary dystonia patients had decreased SICI on the affected side. This is in line with recent finding of reduced SICI in patients with dystonia caused by lentiform nucleus lesions (Trompetto et al.,

2012). Nevertheless, the pathophysiological significance of reduced intracortical inhibition in dystonia remains obscure (Berardelli et al., 2008). Reduced SICI is not specific for dystonia and is found in other BG diseases, including PD and Tourette syndrome (Ridding et al., 1995a, Ziemann et al., 1997) and is also found in clinically unaffected carriers of DYT1 gene mutations (Edwards et al., 2003). Therefore, a loss of intracortical inhibition may be regarded as a non-specific maladaptive change within the motor cortex, caused by chronically disorganised BG output. Our finding would fit into this hypothesis, since SICI was only abnormal on the clinically affected side of our patients with secondary dystonia. It is also possible that reduced SICI arises as a consequence of maintaining an abnormal dystonic posture that could have triggered cortical reorganisation through aberrant afferent input (Espay et al., 2006).

The present data showed only a non-significant trend towards reduced SICI in patients with primary dystonia. Other studies have also reported normal SICI in primary dystonia (Rona et al., 1998, Brighina et al., 2009). This probably reflects the enormous between-subject variability of intracortical inhibition present even in healthy subjects (Wassermann, 2002).

There was no significant difference in the CSP duration between groups, although there was a tendency toward a shortening of the CSP on the affected side in both secondary and primary dystonia, compared to controls. The literature on CSP in dystonia has been less consistent than for SICI, with

studies reporting normal CSP (Stinear and Byblow, 2005) or reduced CSP (Chen et al., 1997) or an abnormality was restricted to a specific task (Tinazzi et al., 2005). SICI and the CSP are thought to depend on GABAa and GABAb cortical interneurons respectively and therefore could be differentially affected by disease (Werhahn et al., 1999, Di Lazzaro et al., 2006, Hallett, 2011). This might explain the abnormal SICI and normal CSP in our secondary dystonia patients. Trompetto et al. (2012) suggested that CSP is reduced in secondary dystonia when the lesion is restricted to the striatum, while it might be normal if the lesion involves the pallidum or thalamus. We did not find the duration of CSP to be related to the anatomical site of the lesion.

Limitations of the study

We acknowledge the limitations of our study. Our sample of secondary dystonia patients is heterogeneous regarding etiology and anatomical site of the lesion. Although it is possible that different lesions could have different functional effect on the motor cortex plasticity, we believe this is unlikely given the similar response to PAS among all secondary dystonia patients, including the lack of spread into the non-target ADM muscle. Another limitation of our data is the long interval between the brain injury and the TMS study. With the present design, we cannot exclude the possibility that motor cortex plasticity was affected at the time of emergence of dystonia, and then over time has reverted to normal. This issue could be addressed in a prospective study that would

need to include a large number of patients, given that only a small proportion of patients with subcortical lesions will go on to develop dystonia.

Conclusion

We have demonstrated that primary and secondary dystonia do not share the same pattern of electrophysiological abnormalities. In secondary dystonia caused by structural brain lesions, the response to PAS is normal, and therefore abnormally enhanced sensorimotor cortical plasticity is not required for the clinical expression of dystonia. In addition, cerebellar function as measured by EBCC is not affected in secondary dystonia, indicating that functional involvement of cerebellum is not an universal feature of dystonia. Our findings may give some insight into why the stimulation-based therapeutic interventions which are thought to interfere with motor cortex plasticity, such as repetitive TMS and DBS, might not be as useful in secondary as in primary dystonia patients (Andrews et al., 2010, Vidailhet et al., 2012). Further exploration of the difference in pathophysiological mechanisms in different types of dystonias may have implications in selecting the most appropriate treatment among different alternatives and also for developing new therapeutic strategies.

Chapter 7 **General Discussion and Conclusion**

In the present series of studies TMS was used to investigate cortical pathophysiology in two common movement disorders, PD and dystonia. The sensorimotor cortex was not considered to be a passive translator of abnormal BG input into aberrant motor output, but rather as a place that could tune the various inputs it receives before providing a final output for movement. Importantly, it was considered that in each disorder, the sensorimotor cortex could take on a dynamic role, going through different levels of functional reorganisation depending on many factors including: disease stage, disease type (i.e. primary vs. secondary dystonia) as well as the functional state of other nodes in the motor network such as the cerebellum and afferent input. With this approach, any changes in electrophysiological measures at the level of sensorimotor cortex could be viewed as either compensatory, maladaptive or as an epiphenomenon of no significance for the disease. This thesis concentrates on the changes the in response to experimental plasticity protocols, given the reputed role of brain plasticity in adaptation to various physiological and pathological inputs.

It has been classically considered that sensorimotor cortical plasticity is reduced or absent in PD (Ueki et al., 2006, Udupa and Chen, 2013), while it is increased

in dystonia (Quartarone et al., 2003, Quartarone and Pisani, 2011). The present series of experiments provides evidence that this is not necessarily always the case.

By using the natural model of clinical asymmetry in PD, we have shown that cortical plasticity changes from being increased early in the course of the disease (and perhaps in the preclinical stage), to being smaller than normal as the disease progresses further. Increased sensorimotor cortical plasticity probably represents a compensatory response, which slows down the appearance and progression of motor signs. This is suggested by the association between the decrease in interhemispheric asymmetry of the PAS response and decrease in motor asymmetry of disease. Thus, early in PD changes in cortical plasticity are probably adaptive. The reduced responsiveness of the sensorimotor cortex to plasticity protocols in advanced PD most likely reflects the deficit of dopamine, which is an important neuromodulator of both LTP and LTD.

We have shown that increased plasticity is not a “sine qua non” of dystonias. Secondary dystonias caused by BG lesions do not manifest enhanced responses to experimental plasticity protocols. This has several implications. Firstly, enhanced plasticity is not a pathophysiological feature of all dystonias but may be a trait only of the primary dystonias. Secondly, changes in cortical plasticity are not needed for the clinical expression of dystonic symptoms

whether they arise as a consequence of cortical reorganisation secondary to abnormal BG output or are secondary to dystonic activity at the periphery, as suggested by normal plasticity in secondary dystonia patients. Moreover, even when present, plasticity changes are not fixed but can be modified by manipulating various other inputs to the sensorimotor cortex. This explains why modifying afferent input with BT injections in cervical dystonia reduces excess plasticity. There are other examples in the literature demonstrating changes in plasticity with changes in BG cortical input after DBS of GPi (Tisch et al., 2007, Ruge et al., 2011).

A second pathophysiological difference between secondary and primary dystonia is in the presence of cerebellar involvement. Cerebellar function as measured by EBCC is not affected in secondary dystonia caused by BG lesions, indicating that functional involvement of cerebellum is not a universal feature of dystonia. However, the on-going debate as to whether changes in cerebellar activity in primary dystonia are (a) compensatory or (b) an epiphenomenon occurring secondary to abnormal activity elsewhere within the sensorimotor network or (c) are a primary part of the pathophysiology of dystonia (Teo et al., 2009, Sadnicka et al., 2012) is still not resolved. The compensation hypothesis is based on the idea that cerebellar hyperactivity, as seen in functional brain imaging of patients with primary dystonia can compensate for abnormalities in motor cortical plasticity. It is supported to some extent by the fact that in healthy subjects' alterations of cerebellar activity using

transcranial direct current stimulation reduces responsiveness to a subsequent PAS protocol (Hamada et al., 2012b). In primary dystonia this compensatory activity may have deleterious effects on sensitive tests of cerebellar function, such as eye-blink conditioning, even though clinical signs of cerebellar dysfunction are absent. This would fit with our finding that since there are no abnormalities in motor cortical plasticity in secondary dystonia, there is no need for compensatory cerebellar activity, and thus EBCC is normal. Nevertheless, the data also could fit into the alternative hypothesis that cerebellar abnormalities are an intrinsic feature of primary dystonia, since they are absent in secondary cases.

7.1. Further studies

Though of interest in their own right, the findings here open up options for further studies into the mechanism of plasticity changes in PD and dystonia and importantly, highlight the therapeutic potential of TMS in both disorders

7.1.1 Delineating the mechanism of increased cortical plasticity in PD

Our data support the role of asymmetric hemispheric reorganisation in compensation for PD, however further studies should address the mechanism through which this might have been occurring.

1) Asymmetric functional cortical reorganisation in early PD may be a consequence of asymmetric BG output from the less and more affected sides. This issue could be addressed in longitudinal studies of clinically still unaffected individuals who are at a high risk of developing PD, such as carriers of PD genes that have a high penetrance. In the potential study, changes in plasticity over time could be correlated with functional neuroimaging of the BG, such as FDG-PET and/or DAT-SCAN.

2) An additional mechanism responsible for the asymmetry of LTP-like plasticity in early PD could be related to abnormal interhemispheric connectivity, consistent with the model of interhemispheric competition in the motor system. This model has been investigated in detail in stroke patients and implies that the affected hemisphere is disabled both by its own damage and by stronger inhibition from the unaffected hemisphere (Murase et al., 2004, Ward and Cohen, 2004). Extending the model of interhemispheric competition to cover our findings, reduced inhibition from the more to the less affected hemisphere could account for increased motor cortex plasticity in the less affected hemisphere. There is previous evidence for reduced transcallosal inhibition in PD, as demonstrated by shortening of the ipsilateral silent period (Priori et al., 1994) and reduced interhemispheric inhibition from the more to the less affected hemisphere in PD patients with mirror movements (Li et al., 2007, Spagnolo et al., 2013). However, no studies have so far investigated if interhemispheric

projections from M1 may affect LTP-like plasticity in the contralateral target hemisphere.

3) A compensatory role of the cerebellum has been suggested in PD (Ballanger et al., 2008, Wu and Hallett, 2013). Recent work has shown that in healthy subjects, sensorimotor cortical plasticity as measured by the response to a facilitatory PAS protocol depends on the functional state of cerebellar output (Hamada et al., 2012b, Popa et al., 2013). If the response to PAS is modulated by inputs from the cerebellum, then an increase in cortical plasticity may be driven by potential compensatory influences from the cerebellum. This may be further investigated in early clinically asymmetric PD patients by studying how the response to PAS in each hemisphere changes after preconditioning the ipsilateral cerebellum with stimulatory and inhibitory TMS protocols.

7.1.2 Therapeutic use of TMS in PD

If increased sensorimotor cortical plasticity of the less affected hemisphere in early PD is an adaptive change that slows down progression of the motor signs, then further enhancing plasticity using non-invasive brain stimulation techniques may result in additional benefits for patients. This may be achieved by applying repeated sessions of stimulatory TMS protocols over the less affected side of early PD patients or even in the preclinical phase of disease. It would also be

interesting to investigate if the response to rTMS depends on the treatment status of the patients. In our study, we have not shown that early initiation of dopaminergic treatment affects the change in the PAS response that occurs with disease progression, however our sample was small and our study was not primarily designed to answer this question.

7.1.3 Further defining the role of cortical plasticity in dystonias

Previous work (Quartarone et al., 2003, Weise et al., 2006) and our study provide evidence that plasticity is abnormal in the primary dystonias, while we show that plasticity is normal in patients with secondary dystonia due to BG lesions, as it is in patients with psychogenic dystonia (Quartarone et al., 2009). This suggests that functional cortical involvement differs between various forms of dystonia. Further studies may address whether cortical plasticity and cerebellar function are involved in some other dystonias, such as drug-induced dystonias or Dopa-responsive dystonias. This could help to resolve the debate on whether there is a common pathophysiological model for all the dystonias or rather different forms of dystonia may have different faulty neuroanatomical networks.

Furthermore, as all electrophysiological studies on primary dystonias have dealt with patients who have had at least several years history of dystonic symptoms, it is still not known if abnormal plasticity is present at the initial presentation of

the disease, as would be expected if enhanced plasticity is indeed pathophysiological trait that predisposes individuals to developing symptoms.

Finally, we have shown that injection of BT injections temporarily reduces plasticity for a short period after the injections (Chapter 5), while it remains to be studied if chronic BT treatment affects plasticity in the long term.

7.2 Methodological “lesson”: resolving controversies

Increasing numbers of TMS studies in PD and dystonia have contributed to the progress in the electrophysiological characterization of BG diseases. Nevertheless, assometimes quite opposite findings have been recorded in the same disease, confusion has occurred when interpreting the pathophysiological findings. For example, in PD, the response to PAS is typically reported as being reduced (Morgante et al., 2006, Ueki et al., 2006, Kawashima et al., 2013), but some studies have found the opposite, namely an increased response to PAS (Bagnato et al., 2006). Opposite findings exist also for iTBS induced cortical plasticity in PD (Suppa et al., 2011, Zamir et al., 2012). In dystonia, intracortical inhibition as measured by SICI or CSP has been reported as either reduced or normal (Hallett, 2011). These controversies can be explained by the details of the experimental protocols and also by the characteristics of the patients being studied. Although the average picture of electrophysiological characteristics in PD and dystonia (and other BG disease) may be best revealed by methodological studies on large-scale heterogeneous populations, we believe

that our four studies contribute to resolving some of the controversies, and have some important methodological implications:

1) Our results show that the clinical asymmetry of PD is reflected in asymmetry of cortical TMS parameters. This fact might be one of the reasons for the conflicting findings in previous studies which did not take into account the side of symptom onset. The problem may be avoided if all the patients included in a study are tested on either more or the less affected side.

2) By longitudinally studying the same PD patients, we have shown that electrophysiological abnormalities are not fixed, but rather change with disease progression. This implies that the disease duration can affect TMS measurements, thus contributing to the variability of results. It is therefore important that patients included in TMS studies have similar duration of parkinsonian symptoms.

3) In dystonia patients, the response to an experimental plasticity protocol correlates positively with the time from previous BT injections. The implication is that in studies of dystonia, patients should all be treated with BT at the same time before commenicng the experiments.

4) Different forms of dystonia do not share the same pattern of electrophysiological abnormalities. We have shown differences between secondary and primary dystonia patients, and there is one study providing similar evidence for differences between genetic and non-genetic causes of primary dystonias (Sadnicka et al., in preparation). Therefore, lumping patients with different types of dystonia together should better be avoided, as this may lead to heterogeneous results and add the confusion rather than increasing the knowledge of the pathophysiology of the dystonias.

7.3. General limitations of the studies

There are limitations associated with statistical analysis used, which I will avoid in my future studies. For example, the possibility of type I statistical error ("false positive") may be minimized using Bonferonni corrections for multiple post-hoc comparisons and correlations. Type II statistical error ("false negative") may be minimised if an optimum sample size is based on the power calculations for the expected responses from the previously published studies. I will also report the statistics and the p values of the non-significant results. Finally, I will express variability of the results not only in terms of measures of the central tendency (average values) +/- standard error of the mean, but also in terms of confidence intervals and effect size. This would allow not only more precise estimations of

how much the average values of the clinical and electrophysiological variables are likely to fluctuate but will also permit the results to be included in the future metanalysis studies.

7.4 Conclusions

This work is focused on two intriguing movement disorders, PD and dystonia, in which functional reorganisation of the sensorimotor cortex seems to have an important pathophysiological role. Our approach was to study cortical plasticity and intracortical inhibition in these disorders, and in particular, the relation of these measures with other disease related factors, including duration of disease, etiology, and the impact of medical treatment..

We found that PD is initially characterised by an increase in sensorimotor plasticity, which probably reflects adaptive process that compensate for the presence of motor symptoms. However, with time plasticity decreases as this compensation is lost and the motor symptoms evolve further. Thus, a potential treatment approach to PD could be to intervene in a way that sustains or further increases sensorimotor cortical plasticity, aiming to slow down the progression of the motor signs.

In dystonia, enhanced plasticity seems to represent a maladaptive trait of primary but not secondary forms of the disease. This may explain the well-

known differences in their responses to treatments, as well as providing a rational basis for further therapeutic approaches. Non-invasive brain stimulation techniques that interfere with plasticity might be more appropriate in primary dystonias than in patients with secondary dystonias caused by structural brain lesions.

In conclusion, brain plasticity should be understood as a process of constant adjustment to various situations, wheteher or not these are good or bad, short-lived or enduring. As a consequence of this continuous process, manifestations of neurological disorders may not only depend on the impact of the disease pathology, but also on the brain's potential to undergo plastic changes, adaptive or maladaptive. And even though we may be born with our brain having pre-determined potential to undergo plastic changes, there may still be a prospect of interfering with plasticity for the good of a patient. With this in mind, the first step is to disentangle the functional significance of plasticity changes in neurological disorders, as this will eventually enable us to modify them in a desirable direction, inhibiting changes that lead to disease manifestation and enhancing those that help the patient.

APPENDIX 1: Publications during PhD period related to this thesis

Kojovic M, Pareés I, Kassavetis P, Palomar FJ, Mir P, Teo JT, Cordivari C, Rothwell JC, Bhatia KP and. Edwards MJ .Secondary and primary dystonia: pathophysiological differences. Brain. 2013 Jul;136(Pt 7):2038-49.

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Bologna M and **Kojovic M.** Interfacing basal ganglia models and Parkinson's disease phenomenology: how can we translate the findings of electrophysiological studies from research to clinic? Basal Ganglia, 2 (2012) 189-193

Kojovic M, Edwards MJ, Parees I, Rothwell JC, Bhatia KP. Secondary cervical dystonia caused by cerebellar cystic lesion--a case study with transcranial magnetic stimulation. Clin Neurophysiol. 2012 Feb;123(2):418-9.

Kojovic M, Caronni A, Bologna M, Rothwell JC, Bhatia KP, Edwards MJ. Botulinum toxin injections reduce associative plasticity in patients with primary dystonia. Mov Disord. 2011 Jun;26(7):1282-9.

APPENDIX 2: Other publications during PhD period

Pareés I, **Kojovic M**, Pires C, Rubio-Agusti I, Saifee TA, Sadnicka A, Kassavetis P, Macerollo A, Bhatia KP, Carson A, Stone J, Edwards MJ Physical precipitating factors in functional movement disorders.J Neurol Sci. 2014 Jan. doi: 10.1016/j.jns.2013.12.046. [Epub ahead of print]

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