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HIGH FREQUENCY REPETITIVE SENSORY STIMULATION IN HEALTHY SUBJECTS AND DYSTONIA

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High Frequency Repetitive Sensory Stimulation in Healthy Subjects and Dystonia – Roberto Erro

PhD thesis

Verona, 17/05/017

DECLARATION THAT THE WORK PRESENTED IN THIS THESIS IS THE CANDIDATE'S OWN

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

Verona, May 2017

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ABSTRACT

This thesis describes a series of studies involving both healthy subjects and patients with dystonia, in which the mechanisms of inhibitory plasticity have been explored with the use of a novel non-invasive brain stimulation technique, namely High-Frequency Repetitive Sensory Stimulation (HF-RSS), to understand how inhibitory mechanisms contribute to the pathogenesis of dystonia.

To this aim, several "preliminary" and parallel experiments have been conducted to fully characterize the neurophysiological abnormalities in dystonia and the physiological changes induced by HF-RSS in healthy subjects. Thus, I have explored:

1. The neurophysiological correlates of abnormal somatosensory temporal discrimination in cervical dystonia, linking this behavioural abnormality with defective inhibitory mechanisms within the sensory cortex;
2. The behavioural consequences of HF-RSS in healthy subjects in terms of somatosensory temporal discrimination, showing that this technique can be in fact used as a novel non-invasive brain stimulation protocol in order to reversibly improve somatosensory temporal discrimination;
3. The neurophysiological mechanisms by which the observed behavioural improvement occurs after HF-RSS in healthy subjects. Thus, the improvement of somatosensory temporal discrimination is mostly driven by an enhancement of inhibitory processes occurring within the primary sensory cortex, a phenomenon known as inhibitory plasticity;

4. Whether HF-RSS could ameliorate inhibitory processes in cervical dystonia and, in turn, lead to an improvement of somatosensory temporal discrimination. It is here shown that patients showed a paradoxical response to such a stimulation protocol, suggestive of defective inhibitory plasticity as one of the main mechanisms contributing to the pathogenesis of dystonia.

These results contribute to the understanding of the pathophysiology of dystonia, opening a novel window for future research and possibly novel treatments. Moreover, these results widened the understanding relative to this novel type of non-invasive brain stimulation that can be theoretically used for the study of other disorders where central inhibitory processes are thought to be defective.

SOMMARIO

Questa tesi descrive una serie di esperimenti su soggetti sani e pazienti con distonia, in cui sono stati studiati i meccanismi di plasticitá inibitoria tramite l'utilizzo di una nuova tecnica di neuromodulazione non-invasiva chiamata "Stimolazione ripetitiva sensitiva ad alta frequenza" (HF-RSS) allo scopo di capire come i meccanismi d'inibizione a livello cerebrale contribuiscano alla patogenesi della distonia.

Con questo fine, diversi studi "preliminari" sono stati condotti in parallelo per caratterizzare a pieno le alterazioni neurofisiologiche nei pazienti con distonia e le modifiche fisiologiche indotte da tale tecnica nei soggetti sani.

A tal fine, ho esplorato:

1. I correlati neurofisiologici della discriminazione temporale somatosensoriale nei pazienti con distonia, correlando le alterazioni della disciminazione temporale con un'alterazione dei meccanismi inibitori a livello della corteccia sensitiva primaria;
2. Gli effetti della stimolazione ad altra frequenza (HF-RSS) a livello psicofisico, mostrando come questa stimolazione possa effettivamente essere utilizzata come protocollo di neuromodulazione non-invasivo per migliorare la discriminazione temporale somatosensoriale;
3. I meccanismi neurofisiologici che spiegano il miglioramento della discriminazione somatosensoriale dopo la stimolazione, mostrando che tale miglioramento é dovuto ad un potenziamento dei meccanismi inibitori intracorticali, un fenomeno noto come plasticitá inibitoria;
4. Se tale stimolazione ad alta frequenza potesse potenziare i meccanismi inibitori in pazienti con distonia cervicale e, di conseguenza, migliorare la discriminazione temporale

somatosensoriale: Ho quindi dimostrato dimostrando che i pazienti hanno una risposta paradossa a tale protocollo di stimolazione. Tale risultato suggerisce che la plasticitá inibitoria é intrinsecamente anomala e rappresenta uno dei principali meccanismi della patogenesi nella distonia.

Questi risultati contribuiscono ad ampliare le conoscenza sulla patogenesi della distonia e aprono una nuova finestra di ricerca, individuando un nuovo target, eventualmente passibile di trattamento. Inoltre, questi risultati hanno espanso le conoscenza relativa a questo nuovo tipo di neuromodulazione non-invasiva, suggerendo che essa potrebbe essere utilizzata per lo studio di altre patologie del sistema nervoso in cui si sospetta una riduzione o un'anomalia dei meccanismi che regolano l'inibizione a livello centrale.

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TABLE OF CONTENTS

Abstract……4

Sommario……6

Acknowledgements……8

Table of contents……9

Chapter 1. General introduction to the pathophysiology of dystonia……12

1.1. Definition of dystonia……12

1.2. The pathophysiology of dystonia……13

1.2.1 Loss of inhibition……13

1.2.2 Excessive (maladaptive) plasticity……15

1.2.3 Deranged sensorimotor integration……16

1.3 Sensory processing deficits in dystonia……18

1.3.1 A brief overview of sensory abnormalities in dystonia..18

1.3.2 Somatosensory temporal discrimination threshold……23

Chapter 2. The concept on non-invasive brain stimulation for dystonia....25

2.1 General overview of NIBS in dystonia……25

2.2 High Frequency repetitive sensory stimulation……27

Chapter 3. Aims and hypotheses……30

Chapter 4. Methods……32

4.1 Somatosensory temporal discrimination threshold……32

4.2 Somatosensory evoked potentials recording and analysis……33

4.3 Transcranial magnetic stimulation and electromyographic recording……35

4.4 High frequency repetitive sensory stimulation……36

Chapter 5. Neurophysiological correlates of abnormal STDT in cervical dystonia……38

5.1 Introduction……38

5.2 Methods……39

5.2.1 Participants……39
5.2.2 Procedure……39
5.2.3 Statistical analysis……40
5.3 Results……40
5.4 Discussion……43
Chapter 6. High frequency repetitive sensory stimulation reversibly improves STDT in healthy subjects……48
6.1 Introduction……48
6.2 Methods……49
6.2.1 Participants……49
6.2.2 Procedure……49
6.2.3 Statistical analysis……50
6.3 Results……50
6.4 Discussion……52
Chapter 7. High frequency sensory stimulation increases sensorimotor inhibition in healthy subjects……55
7.1 Introduction……55
7.2 Methods……57
7.2.1 Participants……57
7.2.2 Procedure……57
7.2.3 Statistical analysis……57
7.3 Results……58
7.3.1 Somatosensory discrimination threshold……58
7.3.2 N20 and P14 latency and amplitude……59
7.3.3 SSEP recovery cycle and HFO……60
7.3.4 Correlations between behavioural and neurophysiologic measures……63
7.3.5 Effects of HF-RSS on M1 inhibitory circuitry……64

7.4 Discussion……66
Chapter 8. Abnormal inhibitory plasticity in cervical dystonia……69
8.1 Introduction……69
8.2 Methods……70
8.2.1 Participants……70
8.2.2 Procedure……70
8.2.3 Statistical analysis……70
8.3 Results……71
8.3.1 Somatosensory Discrimination Threshold……71
8.3.2 Somatosensory Evoked Potentials……71
8.3.3 Sensory lateral inhibition……73
8.3.4 SSEP recovery cycle……75
8.3.5 SEEP high frequency oscillations……75
8.3.6 Corticospinal excitability……76
8.3.7 Cortical inhibition in the motor system……77
8.3.8 Correlations……79
8.4 Discussion……80
Chapter 9. Conclusions and future directions……87
9.1 HF-RSS is a novel NIBS protocol able to induce inhibitory plasticity……87
9.2 Inhibitory plasticity is defective in cervical dystonia……89
List of figures……92
List of tables……95
Abbreviations……96
Peer-reviewed publications……98
References……101

Chapter 1:

General introduction to the pathophysiology of dystonia

1.1 Definition of dystonia

Dystonia is a syndrome characterized primarily by excessive muscle contractions giving rise to abnormal posture and involuntary twisting movements (Albanese et al., 2013). Dystonia can be classified in a number of ways, according to the age-at-onset, distribution, presence of additional signs, and aetiology. The current classification relies on two axes: the first defines the clinical features and phenomenology of dystonia in any given patient, whereas the second addresses etiological factors (Albanese et al., 2013). In most patients, however, definitive aetiological conclusions cannot be reached and the dystonia syndrome is hence referred to as idiopathic.

The term *dystonia* has been used both to describe the hyperkinetic movement disorder itself and to embrace a group of disorders in which dystonia may be the only sign, or part of a syndrome. The classification of dystonia according to its distribution is commonly used in the medical literature (whenever referring to idiopathic forms) and will be hence adopted in the current thesis. This approach stems from the concept that patients with a similar phenotype, for example cervical dystonia (CD), would share the same pathophysiology. There are in fact several demographic and clinical features that differentiate CD patients from patients with other forms of dystonia, for instance focal hand dystonia (FHD), suggesting that these represent distinct "disease entities" (Erro et al., 2014).

1.2 The pathophysiology of dystonia

Despite dystonia being a widely heterogeneous group of disorders, certain pathophysiological mechanisms have been consistently identified across different forms of idiopathic dystonia. Thus, three main neurophysiological abnormalities have been construed to represent the pathophysiological substrate of dystonia: loss of inhibition at different levels of the CNS, maladaptive (excessive) plasticity, and altered sensorimotor integration (Quartarone & Hallett, 2013).

1.2.1 Loss of inhibition

Patients with dystonia have a widespread loss of inhibition that has been first demonstrated in spinal [i.e., loss of reciprocal inhibition in the arm of patients with FHD (Panizza, Lelli, Nilsson, & Hallett, 1990)] and brainstem [i.e., blink reflex recovery cycle in patients with blepharospasm (BPS) (Berardelli, Rothwell, Day, & Marsden, 1985)] reflexes. These abnormalities have subsequently found in patients with generalized dystonia (Tisch, Limousin, Rothwell, Asselman, Quinn, et al., 2006; Tisch, Limousin, Rothwell, Asselman, Zrinzo, et al., 2006) and likely reflect abnormal supraspinal control signals. Such a loss of reciprocal inhibition could partly account for the co-contraction of antagonist muscles that characterizes voluntary movement in dystonia (Hallett, 2011).

Loss of inhibition can also be demonstrated within the motor cortex with a variety of electrophysiological techniques, each of which evaluates a specific inhibitory circuit, most within the cortex itself. These inhibitory circuits include at least one class of inhibitory interneurons, and it is possible that some of these methods might tap some of the same interneurons. Thus, short intra-cortical inhibition (SICI), which is largely mediated by GABA-A receptors (Di Lazzaro et al., 2000), is reduced in FHD (Ridding, Sheean, Rothwell, Inzelberg, & Kujirai, 1995). This reduction was

observed in both hemispheres of patients suggesting that this abnormality more likely reflects a substrate for dystonia: neither is sufficient on its own to determine clinical manifestations nor is a mere consequence of the dystonic symptoms. Reduced SICI has been subsequently confirmed as one of the commonest abnormalities in dystonia in most (Espay et al., 2006; Y. Z. Huang, Rothwell, Lu, Wang, & Chen, 2010; McDonnell, Thompson, & Ridding, 2007) but not all (Brighina et al., 2009; Stinear & Byblow, 2004) studies.

The cortical silent period (CSP) is another electrophysiological marker of cortical inhibition that is represented by a pause in ongoing voluntary electromyography (EMG) activity produced by a single pulse of transcranial magnetic stimulation (TMS) (Fuhr, Agostino, & Hallett, 1991). This type of inhibition, especially in its latter part, is likely mediated by GABA-B receptors (Werhahn, Kunesch, Noachtar, Benecke, & Classen, 1999). In fact, SICI and the CSP show different modulation and clearly reflect different aspects of cortical inhibition. The CSP is shortened in focal dystonia (Chen, Wassermann, Canos, & Hallett, 1997; Espay et al., 2006; Kimberley et al., 2009) indicative of loss of inhibition, although this was not seen in all investigations (Stinear & Byblow, 2005). Differently from SICI, this deficit may be restricted to the symptomatic hand (Chen et al., 1997) or can be only detected during certain motor activities, suggesting some task specificity for this abnormality (Tinazzi et al., 2005).

An additional marker of intra-cortical inhibition that, as the CSP, likely relies on GABA-B receptors is the long-latency cortical inhibition (LICI). Analogous to the CSP, LICI has been found deficient in the affected hand of patients with FHD (Espay et al., 2006) and only with background contraction (Chen et al., 1997). This abnormality is particularly interesting since it is restricted to the symptomatic setting and, hence, might reflect a correlate of the clinical development of the dystonia (Hallett, 2011).

Bearing in mind that the aforementioned alterations are non-specific in that they have also been demonstrated in various other neurological conditions, reduced intra-cortical inhibition does not appear in itself sufficient to produce dystonia. Some authors also noted that physiological abnormalities in asymptomatic body parts could indicate that they are compensatory changes to prevent dystonia. However, this seems unlikely since these abnormalities are similar to those in the symptomatic body parts and are in the direction to lead to motor dysfunction (Hallett, 2011).

An additional argument that patients with dystonia have defective inhibition comes from the evidence of loss of *surround* inhibition in these subjects. The basic idea of surround inhibition (also referred to as *lateral* inhibition) is that muscles not involved in a specific movement will show active inhibition during the movement. A similar mechanism has been proposed in the sensory domain to allow a more exact perception of incoming sensory information. Both motor and sensory surround inhibition has been demonstrated to be deficient in patients with dystonia (Hallett, 2011; Tinazzi et al., 2000). The exact underpinnings of both remain unknown since this type of inhibition poorly correlates with measures of SICI, CSP and LICI.

Whereas SICI, CSP and LICI reflect mechanisms mostly acting within the motor cortex, other electrophysiological techniques tap the interaction(s) between sensory and motor cortices. As such, despite some of these also reflecting a failure in inhibition, these layers of evidence will be discussed below, in the context of abnormal sensorimotor integration.

1.2.2 Excessive (maladaptive) plasticity

There is large evidence suggesting that both the motor and sensory cortex in dystonia exhibits an exaggerated responsiveness to conditioning protocols able to induce plastic changes. A well-established approach to

test plasticity in humans in a non-invasive way is paired associative stimulation (PAS). Using PAS, it has been demonstrated that both long-term potentiation (LTP)-like and long-term depression (LTD)-like effects on motor responses are enhanced in patients with FHD (Quartarone & Pisani, 2011; Quartarone et al., 2005), yet with a high inter-individual variability (Sadnicka, Hamada, Bhatia, Rothwell, & Edwards, 2014). The enhanced motor responses are not only observed in the target muscle but also in nearby muscles (Quartarone et al., 2005), which is indicative of loss of surround inhibition, as mentioned earlier. In theory, the excessive plasticity in itself might be explained by a reduction of inhibition (Hallett, 2011), but there is no agreement on this with some authors believing excessive plasticity is a *primary* abnormality in dystonia (Quartarone & Hallett, 2013; Quartarone & Pisani, 2011).

The alterations of plasticity might be present at the sensory cortical level, as demonstrated by a single study showing increased amplitude of the P27 component of SSEP in FHD after PAS (Tamura et al., 2009), and also found at the brainstem level. In fact, an excess of plasticity was observed within the blink reflex circuits in patients with BPS (Quartarone et al., 2006).

This abnormal plasticity is not confined to the neural circuits affected by dystonia but is generalized across the entire sensorimotor system (Quartarone et al., 2008), and it has been demonstrated to be abnormal in non-manifesting carriers of *TOR1A* gene mutations (Edwards, Huang, Mir, Rothwell, & Bhatia, 2006), thus potentially representing an endophenotypic trait of dystonia.

1.2.3 Deranged sensorimotor integration

Another theme that has recently gained momentum in the pathophysiology of dystonia is a defect in sensory processing. The hypothesis has been in fact raised that deranged processing of the

somatosensory input may lead to abnormal sensorimotor integration, thus contributing substantially to the generation of dystonic movements (Quartarone & Hallett, 2013; Tinazzi, Frasson, Bertolasi, Fiaschi, & Aglioti, 1999; Tinazzi et al., 2000). While the evidence coming from behavioural studies for sensory abnormalities in dystonia, with a particular focus to the somatosensory temporal discrimination threshold (STDT), will be discussed in the next paragraph, here I describe the electrophysiological evidence for deranged sensorimotor integration in dystonia.

The techniques used to assess sensorimotor integration evaluate how motor responses induced by TMS are influenced by sensory afferents delivered as an electric shock to a peripheral nerve prior to the magnetic pulse. As mentioned earlier, these techniques evaluate other sets of inhibitory circuits, the effects of which can be either at short-latency (SAI – i.e., short afferent inhibition), at about 20 ms, or at long-latency (LAI – i.e., long afferent inhibition), at about 200 ms. Both SAI and LAI can be used to probe homotopic (by stimulating a nerve closely related to the target muscle) or heterotopic (by stimulating a nerve somewhat distant to the muscle) effects. SAI is mediated by both cholinergic (Tokimura et al., 2000) and GABA-A influences (Di Lazzaro et al., 2007) and more likely reflect S1-M1 connections, whereas the mechanisms underneath LAI are less clear and probably involve the basal ganglia and other associative cortical areas.

A study of homotopic LAI at rest showed that patients with dystonia converted inhibition into facilitation, with augmented motor responses (Abbruzzese, Marchese, Buccolieri, Gasparetto, & Trompetto, 2001). This dramatic abnormality was only seen in patients with FHD and not in those with CD, indicating that this abnormality might be specific to the former. In another study involving FHD patients, a deficiency of SAI was observed (McDonnell et al., 2007). However, these abnormalities were not

consistently found in patients with dystonia (Avanzino et al., 2008; Hallett, 2011).

Another approach to evaluate *in vivo* how somatic stimuli interact with motor responses is to combine TMS with low amplitude muscle vibration. When the TMS pulse is delivered over M1 after 1 sec of hand muscle vibration, M1 excitability is physiologically increased in the target (i.e., vibrated) muscle and depressed in adjacent muscles as function of enhanced surround inhibition (Rosenkranz & Rothwell, 2003). In patients with FHD, this pattern of sensorimotor interaction is abnormal and there is only a little effect of vibration on cortical excitability (Rosenkranz et al., 2005).

This body of works, coupled with the imaging and psychophysical evidence of widespread sensory deficits in dystonia as well as with the excessive motor responses following sensory conditioning (as observed after the PAS protocol), corroborated the hypothesis that sensorimotor integration is abnormal in dystonia and plays a substantial role in its pathogenesis (Quartarone & Hallett, 2013).

1.3 Sensory processing deficits in dystonia

1.3.1 A brief overview of sensory abnormalities in dystonia

There are several layers of evidence suggesting that patients with dystonia have several deficits in sensory processing. The initial hypothesis that sensory processing could be disrupted in dystonia stemmed from a primate model of dystonia in which enlarged and overlapped sensory receptive fields were found (Byl, Merzenich, & Jenkins, 1996). Such a finding was later confirmed in humans using EEG, magneto-encephalographic and functional MRI techniques (Bara-Jimenez, Catalan, Hallett, & Gerloff, 1998; Butterworth et al., 2003; Elbert et al., 1998). Electrophysiological studies further corroborated the argument that

sensory abnormalities are present in dystonia (Tamura et al., 2008; Tamura et al., 2009; Tinazzi et al., 2000).

Subsequent to the aforementioned observations, a number of studies aimed therefore to explore sensory abilities in dystonia (Avanzino, Tinazzi, Ionta, & Fiorio, 2015). Table 1.1 provides a summary of the behavioural and psychophysical studies performed in different forms of dystonia, consistently arguing for an abnormal sensory processing in dystonia and consequent aberrant sensorimotor integration.

Function	Task	Type of dystonia	Main results	Fist author, year
Tactile	Spatial Discrimination Task	FHD, CD, BPS, *TOR1A* carriers	Higher threshold in different forms of dystonia than in HC	(Bara-Jimenez, Shelton, & Hallett, 2000; F. M. Molloy, Carr, Zeuner, Dambrosia, & Hallett, 2003; O'Dwyer et al., 2005; Peller et al., 2006; Walsh & Hutchinson, 2007; Walsh et al., 2007; Zeuner et al., 2002)
	Temporal discrimination task	FHD, CD, BPS, *TOR1A* carriers	Higher threshold in different forms of dystonia than in HC	(Aglioti, Fiorio, Forster, & Tinazzi, 2003; Antelmi et al., 2016;

				Bara-Jimenez, Shelton, Sanger, & Hallett, 2000; Bradley et al., 2009; Fiorio et al., 2007; Fiorio, Tinazzi, Bertolasi, & Aglioti, 2003; Fiorio et al., 2008; Kagi et al., 2013; Kimmich et al., 2014; Sadnicka et al., 2013; Scontrini et al., 2009; Tinazzi, Fasano, et al., 2013; Tinazzi et al., 2002; Tinazzi et al., 1999)
	Aristotle's illusion	FHD, CD, BPS	Reduced illusion on the unaffected fingers of the affected hand only in FHD	(Tinazzi, Marotta, et al., 2013)
Proprioceptive	Muscle vibration	FHD, CD	TVR is normal, whereas the perception of real/illusory arm	(Bove, Brichetto, Abbruzzese, Marchese, &

			movements is abnormal	Schieppati, 2004; Frima, Nasir, & Grunewald, 2008; Frima, Rome, & Grunewald, 2003; Grunewald, Yoneda, Shipman, & Sagar, 1997; Rome & Grunewald, 1999; Yoneda, Rome, Sagar, & Grunewald, 2000)
	RHI	FHD, CD	The proprioceptive drift associated to the RHI is reduced in FHD, selectively on the affected hand	(Fiorio et al., 2011)
Sensorimotor integration	Grip-force adjustments	FHD	Impaired visuomotor tracking control and force-matching performance in both hands. Increased grip force in patients than HC	(Bleton et al., 2014; Serrien, Burgunder, & Wiesendanger, 2000)

	Reaching movements	FHD, CD	Impaired upper limb trajectories toward a target	(Marinelli et al., 2011; Pelosin, Bove, Marinelli, Abbruzzese, & Ghilardi, 2009)

Table 1.1 Summary of the behavioural/psycophysical evidence for abnormal sensory processing and/or sensorimotor integration

(Modified from Avanzino et al., 2015)

Abbreviations not present in the abb. list: TVR=Tonic Vibration Reflex; RHI=Rubber Hand Illusion.

Among these abnormalities, STDT would appear the most reliable deficit observed in dystonia and, hence, will be commented on separately in the next paragraph. In summary, available evidence supports the argument that in dystonia deficits extend beyond the motor control and further involve processing of sensory inputs. However, the anatomical and physiological bases of some of these abnormalities are not yet clear and it is currently unknown to what extent sensory abnormalities contribute to the development of dystonia. The proposal that *"misprocessing of sensory feedback coupled with an abnormal excitability within inhibitory motor circuits at different level (spinal cord, brainstem, cerebellum, basal ganglia, and cortex) may result in a progressive abnormal plasticity in local and distant nodes, culminating in an overt dystonia"* (Quartarone & Hallett, 2013) has been put forward, but more experimental evidence is needed to confirm this suggestion.

1.3.2 Somatosensory temporal discrimination threshold

STDT is defined as the shortest time interval necessary for a pair of tactile stimuli to be perceived as separate (Ramos, Esquenazi, Villegas, Wu, & Hallett, 2016). In young healthy individuals, this interval ranges from 30 to 50 milliseconds, but it tends to increase with age, somewhat reflecting the overall physiological, structural, and metabolic changes that occur in the elderly, despite showing significantly less age-dependence than other candidate sensory tests (Ramos et al., 2016).

As mentioned above, STDT has been suggested to be the most reliable marker of sensory processing deficits and further construed to be an endophenotypic trait in dystonia (Avanzino et al., 2015; Fiorio et al., 2003; Fiorio et al., 2008; Tinazzi et al., 2002; Tinazzi et al., 1999; Walsh et al., 2007). This proposal stems from several layers of evidence showing increased STDT in different forms of adult-onset primary dystonia (i.e., CD, BPS, FHD, laryngeal dystonia) as compared to age-matched HC (Bara-Jimenez, Shelton, & Hallett, 2000; Bradley et al., 2009; F. M. Molloy et al., 2003; O'Dwyer et al., 2005; Scontrini et al., 2009; Tinazzi et al., 1999; Walsh et al., 2007). STDT was further found to be abnormal in manifesting and non-manifesting *TOR1A* (DYT1) carriers (Fiorio et al., 2007). Moreover, STDT was shown to be higher in the affected and unaffected body regions with no correlation with disease severity or duration (Bara-Jimenez, Shelton, Sanger, et al., 2000; Bradley et al., 2010; Scontrini et al., 2009; Walsh et al., 2007), and in patients' unaffected first and second degree relatives (Bradley et al., 2009; O'Dwyer et al., 2005; Walsh et al., 2007), suggesting a primary endophenotypic deficit rather than a deficit secondary to the presence of dystonic contractions. Based on the proposed criteria for a putative endophenotype [i.e., it should: 1) be associated with the disease under investigation in the general population; 2) be an heritable trait transmitted with disease in pedigrees; 3) be ''state-

independent'' or, in other words, unaffected by disease expression or treatment; and 4) have a higher frequency amongst unaffected relatives in pedigrees than in the general population], one study directly compared different candidate endophenotypes in dystonia, demonstrating that STDT fulfils criteria for a reliable endophenotype with a high sensitivity, as compared to others such as the spatial discrimination threshold (Bradley et al., 2010).

Chapter 2:

The concept of non-invasive brain stimulation in dystonia

Part of the information presented in this chapter was originally submitted in the form of a review article: Erro R, Morgante F, Tinazzi M, Bathia KP. Non-invasive brain stimulation for dystonia: Therapeutic implications. Submitted.

2.1 General overview of NIBS in dystonia

The current mainstream symptomatic therapy for dystonia is represented by chemodenervation by means of botulinum neurotoxin (BoNT) injections. However, while success rates in patients with CD or BPS are reasonably high, in patients with FHD outcomes are more often disappointing, also due to frequent adverse effects (Karp, 2012; Karp et al., 1994; Lungu, Karp, Alter, Zolbrod, & Hallett, 2011). Moreover, BoNT might not be sufficient when dystonia is distributed over several body regions, as in many children with generalized dystonia. The role of deep brain stimulation (DBS) in dystonia is emerging (Picillo, Lozano, Kou, Munhoz, & Fasano, 2016), but not all patients are suitable candidates. Thus, alternative therapeutic approaches are clearly needed. The putative pathophysiologic mechanisms of dystonia have been exploited for the development of non-invasive brain stimulation (NIBS) techniques able to induce plastic changes in one or more nodes of the altered network and possibly reverse the aforementioned abnormalities (Wagle Shukla & Vaillancourt, 2014). The concept of neuromodulation holds onto the hope of translating such NIBS techniques into novel therapeutic strategies for dystonia (Wagle Shukla & Vaillancourt, 2014).

Currently, two main techniques are available for human NIBS: transcranial magnetic stimulation (TMS) and transcranial current stimulation (tCS). These neuromodulatory techniques are applied non-invasively over the scalp and hence avoid the possible complications associated with DBS

surgery and the side effects of systemic medications (Cho & Hallett, 2016; Quartarone et al., 2014; Tyvaert et al., 2006; Wagle Shukla & Vaillancourt, 2014; Wu, Fregni, Simon, Deblieck, & Pascual-Leone, 2008). Theoretically, both can be applied over selected cortical regions to modulate the specific cortical–subcortical network that is supposedly linked with a given subset of symptoms.

Both techniques can be set in order to produce either an excitatory or inhibitory effect. Thus, considering the loss of inhibition is one of the most important hallmarks in the pathophysiology of dystonia, then augmenting inhibition might theoretically be an useful strategy to relieve dystonic postures. It is beyond the aims of the current thesis to review all studies that employed NIBS techniques in dystonia but, described in general, almost all studies failed to demonstrate a consistent clinical benefit (Cho & Hallett, 2016; Quartarone et al., 2014). The reasons for this might, however, rely on the fact that there is no consensus about the inherent settings of the technique that is used as well as about the "amount" of stimulation needed for an improvement to be seen. Another crucial issue is with regards to the topographic specificity of the stimulation. Some authors have suggested in fact that this lack of topographic specificity might in itself undermine the usefulness of these techniques (Davis & van Koningsbruggen, 2013). Moreover, there is an established tendency of spread from the target brain area to neighboring areas, which made some authors arguing that the term *non-invasive* would be inappropriate (Davis & van Koningsbruggen, 2013). All these issues call for the development of alternative NIBS techniques. As discussed in detail in the next paragraph, a novel stimulation protocol named High Frequency Repetitive Sensory Stimulation (HF-RSS), that is ostensibly different from both TMS and tCS as the stimulation is not delivered over the scalp but peripherally, has been suggested to induce plastic changes and improve sensory perception

in the stimulated area in both animal and human experiments (Dinse et al., 2006; Godde, Berkefeld, David-Jurgens, & Dinse, 2002; Godde, Spengler, & Dinse, 1996; Godde, Stauffenberg, Spengler, & Dinse, 2000). The behavioural consequences of HF-RSS (i.e. improved sensory performance) would suggest this novel stimulation technique might be a useful tool in dystonia.

2.2 High Frequency Repetitive Sensory Stimulation

Recently, a novel paradigm developed by Godde and colleagues has been shown to improve sensory perception in the stimulated area in animal experiments and in humans (Dinse et al., 2006; Godde et al., 2002; Godde et al., 1996; Godde et al., 2000). The protocol consisted of a passive, unattended, tactile stimulation on a time-scale of a few hours or less (Dinse et al., 2006; Godde et al., 2002; Godde et al., 1996; Godde et al., 2000). What would make this protocol different from other NIBS techniques is that the stimulation is delivered peripherally (for instance, from a digit), the inputs travelling through the physiological sensory pathway to target specific cortical (sensory) areas.

The general idea behind the development of this protocol was based on the evidence suggesting the importance of temporally correlated inputs in the induction of plastic changes: Hence, the authors first evaluated the effects of variation of input statistics by the use of tactile stimulation through temporally coherent patterns on the cortical reorganization in animals. Thus, in adult rats repeated high frequency stimulation of sensory (electrical) inputs from a digit increased the representational area of that digit in sensory cortex and increased the receptive field size of individual cortical neurons (Godde et al., 1996). It was suggested that stimulation led to ‘‘co-activation’’ of receptive fields underneath the electrodes, and that this induced lasting changes in central sensory representations (Godde et

al., 1996). The “co-activation” nature (i.e., the engagement of different pools of neurones with different RFs) of this type of stimulation was demonstrated using a protocol of identical stimulus pattern applied to only a single (i.e. small) skin site, which revealed no changes of RFs, suggesting that integration of highly correlated *spatio*temporal inputs is necessary for this protocol to induce plastic changes (Godde et al., 1996). Late, arguably NMDA receptor-mediated response components were enhanced in this experiment, suggesting an involvement of glutamatergic synapses in this type of plasticity (Godde et al., 1996).

To address the question of the relevance of this plastic reorganization at the behavioural level without providing any types of perceptual reinforcement, Godde et al. set up a parallel experiment to test in humans psychophysically the impact of an analogous stimulation protocol by measuring spatial discrimination performance using the “two-point discrimination task” (TPDT) (Godde et al., 1996). It was hence demonstrated that such a protocol could improve perceptual performance, as demonstrated by reduced TPDT (Godde et al., 1996). The same group carried on a number of subsequent experiments with HF-RSS, the methodology of which will be detailed in the next chapter, showing that significant improvement in discrimination performance was reversible within 24 hours and that perceptual changes were highly selective because no transfer of improved performance to fingers that were not stimulated was found (Godde et al., 2000). Moreover, the behavioural improvement was correlated with a significant shift in the localization of the N20-dipole of SSEP obtained from the index finger that was stimulated (Pleger et al., 2001), suggesting that plastic processes related to the improvement were localized in the primary somatosensory cortex and were scaled with the degree of the individual perceptual improvement. In a functional MRI study, it was further demonstrated that the individual

TPDT reduction was linearly correlated with the enlargement of the representational area of that finger in S1, implying a close relationship between improved performance and cortical reorganization (Pleger et al., 2003). Moreover, HF-RSS was shown to induce significant changes of the resting state mu-rhythm in the upper alpha frequency band within distributed sensory and motor cortical areas, suggesting functional connectivity changes (Freyer, Reinacher, Nolte, Dinse, & Ritter, 2012). This evidence is in line with the preliminary evidence that HF-RSS can, more in general, enhance sensorimotor integration and improve motor performances in the elderly (Kalisch, Tegenthoff, & Dinse, 2008, 2010).

As discussed in more detail in the next chapters, the finding of improved perception along with increased representational areas was somewhat surprising since an initial expectation would have been that increased representational areas and/or RFs would reduce tactile acuity. However, as the authors have acknowledged perceptual ability does not necessarily relate to the receptive field size of individual neurons, but instead reflects the sum total of information present in the discharge of many neurons (Dinse, 2006; Godde et al., 2002; Godde et al., 1996; Godde et al., 2000). Yet, the argument that the perceptual improvement is only attributable to plasticity exerted on glutamatergic synapses (i.e. excitatory plasticity) would not be entirely convincing and in fact, as discussed later, one of the aims of the current thesis was to fully investigate the mechanisms whereby such a protocol can enhance perceptual abilities.

Chapter 3:
Aims and Hypotheses

The background above provides a picture about the main pathophysiological themes in dystonia research and emphasizes the hypothesis that sensory deficits are one of the crucial pathophysiological abnormalities in dystonia, arguably representing the substrate that combined with other, yet unknown, factors might predispose to the development of overt dystonia.

Furthermore, it briefly illustrates the concepts underpinning the notion that NIBS techniques can be used for studying the pathophysiology of dystonia, with the hope that these protocols can be ultimately translated into therapeutic tools to implement in clinical practice. In this context, HF-RSS appears an interesting technique to explore, since it would induce plastic changes in the sensory cortex and improve those sensory abilities that are indeed defective in patients with dystonia. However, there was a relatively scarce amount of information regarding the neurophysiological underpinnings accounting for HF-RSS induced behavioural outcomes in healthy subjects and the argument that LTP-like changes on excitatory synapses would justify the perceptual gain was not entirely convincing.

Therefore, I conducted a number of "preliminary" and parallel studies to implicate inhibitory plasticity as one of the main consequences of HF-RSS and build up the case for HF-RSS to be explored in dystonia. Accordingly, I addressed the following questions:

1) Which are the neurophysiological mechanisms accounting for abnormal STDT in dystonia? I hypothesized that this abnormality relies on defective inhibitory mechanisms in cortical sensory areas;

2) Can HF-RSS improve STDT in healthy subjects? Available literature on HF-RSS was only focused on sensory discrimination in the spatial domain. Being STDT the most reliable endophenotype of dystonia, I explored whether HF-RSS could modulate sensory perception in the temporal domain.
3) Which are the neurophysiological correlates of such a perceptual improvement in healthy subjects? I hypothesized that an augmented effectiveness of cortical inhibitory mechanisms (i.e. inhibitory plasticity) would account for the behavioural improvement.
4) Can HF-RSS be used for reverting such sensory abnormalities as higher STDT in patients with dystonia? The experiments performed to address the questions listed above constituted the rationale to test HF-RSS in dystonia. Two alternative results could be predicted for the experiment involving dystonia patients: i) The amount of inhibition is altered in dystonia but inhibitory plasticity is not and, thus, HF-RSS will augment inhibition and lead to perceptual improvement, as in healthy subjects; or ii) The amount of inhibition is altered along with inhibitory plasticity in dystonia, so that patients will paradoxically respond to HF-RSS with a detrimental effect on perceptual abilities.

Chapter 4:
Methods

Since the electrophysiological techniques that have been used in the current work are largely shared across different experiments (described accordingly in different chapters of this thesis), I am here providing a detailed description of the overall methods, with the hope of simplifying the reading of this thesis. The subjects involved in each experiment will be detailed in the corresponding chapter and the methodology briefly recapitulated. It is anticipated that, being the chapters of this thesis highly intertwined, some concepts and implications regarding these techniques will be reiterated, whenever necessary, in different chapters with the deliberate intention of making each of them readable and sustainable in its own right.

4.1 Somatosensory Temporal Discrimination Threshold

STDT was tested administering paired electrical stimuli, starting at an ISI of 0 ms (simultaneous pair) and progressively increasing the ISI in steps of 10 ms (Conte et al., 2016; Conte et al., 2014; Rocchi, Conte, et al., 2016; Tinazzi et al., 2014). Stimuli consisted of square-wave electrical pulses applied with a constant current stimulator (Digitimer DS7A) through surface skin electrodes, with the anode located 0.5 cm distally to the cathode. The right index finger, right thumb and left index finger were tested in separate sessions. The electrodes were applied on the distal phalanx of the examined finger. For the right index finger, stimulation intensity was obtained by delivering stimuli starting from 2 mA and increasing the current in steps of 0.5 mA; the intensity used for the STDT was the minimal intensity perceived by the subject in 10 of 10 consecutive stimuli (Conte et al., 2010; Rocchi, Conte, et al., 2016). For the other two

fingers, the current intensity was adjusted to match the perceived intensity on the right index finger. Subjects familiarized with the task and achieved a stable performance before STDT testing. During the procedure, they had to verbally report whether they perceived a single stimulus or two temporally separate stimuli. The first of three consecutive ISI at which participants consistently reported two stimuli was considered the STDT. To keep the subject's attention level constant during the test and to minimize the risk of perseverative responses, the STDT testing procedure included "catch" trials consisting of a single stimulus delivered randomly (Conte et al., 2010; Rocchi, Conte, et al., 2016). Each finger was tested three times and the STDT was defined as the average the three obtained values and was entered in the data analysis.

4.2 Somatosensory evoked potentials recording and analysis

SSEP were recorded from scalp Ag–AgCl surface electrodes arranged according to the international 10-20 system of EEG electrode placement (Klem, Luders, Jasper, & Elger, 1999). To record the N20-P25 component the active electrode was placed at CP3 and the reference electrode at Fz, while the P14 component was recorded with the active electrode at Fz and the reference on the contralateral mastoid (Cruccu et al., 2008). Digital nerves of the right index finger were stimulated with a constant current stimulator (Digitimer DS7A) through ring electrodes, with the cathode placed at the base of the first phalanx and the anode placed 2 cm distally (Tinazzi et al., 2000). Monophasic square wave pulses of 200 μsec duration were delivered at 250% of the sensory threshold and at a frequency of 5 Hz. Recordings were collected at a sampling rate of 5 KHz, beginning 20 ms before each stimulus and lasting for 100 ms. Data were band-passed filtered from 3 Hz to 2 kHz (Cruccu et al., 2008).

In a first block 1000 sweeps were averaged and N20 peak latency, N20-P25 peak-to-peak amplitude and P14 baseline-to-peak amplitude were measured. The recording from this block was also used to extract and measure SSEP High Frequency Oscillations (HFO). Thus, the stimulus artefact was removed from -10 to +5 ms to avoid ringing due to filtering (Katayama, Suppa, & Rothwell, 2010). The SSEP wide band signal was band pass filtered digitally (400-800 Hz) and averaged. HFO waveform was divided in two components, early (e-HFO) and late (l-HFO) HFO, separated by N20 peak. Onset of e-HFO and offset of l-HFO were defined as their amplitudes exceeding the averaged background noise level by three standard deviations (Murakami, Sakuma, & Nakashima, 2008; Murakami, Sakuma, Nomura, Nakashima, & Hashimoto, 2008; Murakami, Sakuma, Nomura, Uemura, et al., 2008). e-HFO and l-HFO area was measured and entered into the analysis.

Three more recording blocks of 750 frames each were performed to measure SSEP recovery cycle. Thus, 750 trials were averaged and paired pulses at ISI of 5, 20 and 40 ms were delivered in each block, respectively (Valeriani et al., 2005; Vollono, Ferraro, Miliucci, Vigevano, & Valeriani, 2010). In the frames obtained using paired stimuli, the responses following the second stimulus were obtained by subtracting the SSEP waveform obtained by the first stimulus from the waveform following each double stimulus (Valeriani et al., 2005; Vollono et al., 2010). R5, R20 and R40 were defined as the ratio between the second and the first response at ISI of 5, 20 and 40 ms, respectively.

Finally, 2 more blocks of 750 trials each were recorded, the first stimulating the right thumb only and the second stimulating concomitantly the right thumb and right index finger by giving 2 simultaneous stimuli delivered through 2 constant current stimulators. These two blocks were used to calculate the Spatial Inhibition Ratio (SIR)

of N20-25 and P14; SIR was calculated as the ratio TI/(TII)x100, where TI is the SSEP amplitude obtained by simultaneous stimulation of the thumb and index finger and TII is the arithmetic sum of the SSEP obtained by the individual stimulation of the 2 fingers (Tinazzi et al., 2000).

4.3 Transcranial magnetic stimulation and electromyographic recording

EMG activity was recorded through a pair of Ag/AgCl electrodes placed over the right first dorsal interosseous (FDI), abductor pollicis brevis (APB) and abductor digiti minimi (ADM) muscles in a belly-tendon fashion. Raw signal, sampled at 5 kHz with a CED 1401 A/D laboratory interface (Cambridge Electronic Design, Cambridge, UK), was amplified and filtered (bandwidth 20 Hz–2 kHz) with a Digitimer D 360 (Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK). Data were stored on a laboratory computer for on-line visual display and further off-line analysis (Signal software, Cambridge Electronic Design, Cambridge, UK). To ensure complete target muscle relaxation throughout the experimental sessions we continuously monitored the EMG activity with audio and high-gain visual feedback. TMS was carried out using a Magstim 200 stimulator with a 70mm figure-of-eight coil (Magstim Company Limited, Whitland, UK) which produces monophasic waveform stimuli with pulse width ∼0.1 ms. First, the motor hotspot was found, defined as the site within M1 in which TMS evoked the largest MEP in the APB muscle. Then, we found the resting motor threshold (RMT), active motor threshold (AMT), and the intensity able to elicit motor evoked potentials of approximately 1 mV amplitude from APB muscle (1mV-int), which was later used for test pulses. RMT was defined as the lowest intensity able to evoke a MEP of at least 50 μV in five out ten consecutive trials during rest (Rossini et al., 1994), while AMT was defined as the lowest intensity able to evoke a MEP

of at least 200 μV in five out ten consecutive trials during a 10-15% voluntary contraction of the target muscle (C. C. Huang, Su, & Wei, 2005). SICI was obtained through a paired-pulse TMS, with an ISI of 3 ms between the first, conditioning stimulus and the second test stimulus. The test stimulus was set at 1mV-int, while the conditioning stimulus was set at 70%, 80% and 90% AMT, as to obtain a recruitment curve (Kujirai et al., 1993). Twenty paired stimuli for each different intensity of the conditioning stimuli and twenty single stimuli were delivered in a randomized order. SICI was obtained dividing the amplitude of conditioned MEP by the amplitude of the unconditioned MEP. ICF was obtained in a similar fashion, except that the ISI used was 10 ms and the intensity of the conditioning stimulus was 80% AMT (Kujirai et al., 1993). Twenty paired stimuli were given during the same recording block used for SICI. ICF was obtained dividing the amplitude of conditioned MEP by the amplitude of the unconditioned MEP.

LICI was obtained through a paired-pulse TMS, with an ISI of 100 ms between the first, conditioning stimulus and the second test stimulus. The test stimulus was set at 1mV-int, while the conditioning stimulus was set at 60% RMT (McNeil, Martin, Gandevia, & Taylor, 2011). Twenty paired stimuli were randomly delivered during the same block used for SICI and ICF.

4.4 High frequency repetitive somatosensory stimulation

HF-RSS consisted of 20 Hz trains of square wave electrical pulses of 200 μs duration delivered for 1 s, with 5 s inter-train intervals, for 45 min (Schlieper & Dinse, 2012). This appears the minimum interval of time necessary for inducing plastic changes (Schlieper & Dinse, 2012). Stimuli were delivered with a constant current stimulator (Digitimer DS7A) through surface adhesive electrodes of approximately 1 cm^2 area, with the

anode located on the distal phalanx of the right index finger and the cathode located on the proximal phalanx of the same finger. The intensity of the stimulation was set individually at the highest threshold that subjects could tolerate for the whole period of stimulation, since it was demonstrated that perceptual improvement following repetitive sensory stimulation depends monotonically on stimulation intensity (Schlieper & Dinse, 2012). During the stimulation period, subjects were engaged in a conversation or were reading a book.

Chapter 5:

Neurophysiological correlates of STDT in Cervical Dystonia

The work presented in this chapter was originally published in the form of a research article: Antelmi E, Erro R*, Rocchi L, et al. Neurophysiological correlates of abnormal somatosensory temporal discrimination in dystonia. Mov Disord. 2017;32:141-148. [* joint first author]*

5.1 Introduction

We have seen that somatosensory processing is abnormal in several dystonia subgroups, STDT being the abnormality most commonly reported, even in non-dystonic body regions and in about 50% of unaffected first-degree relatives of dystonic patients (cf. Chapter 1). Although there has been mounting research interest in the exploration of the mechanisms underneath abnormal STDT in dystonia, its underpinnings have remained largely unclear.

One previous study in FHD (Tamura et al., 2008) hinted at the possibility that abnormal STDT could be associated with cortical mechanisms acting within S1, as demonstrated by a significant correlation between STDT scores and the suppression of SSEPs at short ISI, which is reflective of a deficit in somatosensory temporal processing. This argument is further supported by a studies that used cTBS, a protocol able to induce LTD-like changes, over S1 and demonstrated an improvement of STDT in both healthy volunteers and patients with FHD (Conte et al., 2012). Beyond the general interpretation that S1 is critically involved in the processing of the STDT, it is however difficult to understand from the latter study which exact mechanisms were involved into the perceptual improvement. A partial answer would come from a further study involving healthy subjects which also used cTBS over S1 and demonstrated an improvement in STDT along with a reduction of the amplitude of SSEP l-HFO (e.g., indicative of

augmented inhibition), thus linking this behavioural outcome with intracortical inhibitory mechanisms (Rocchi, Casula, Tocco, Berardelli, & Rothwell, 2016).

Based on this previous information, we aimed to explore in-depth the neurophysiological correlates of abnormal STDT in patients with CD, the most common form of adult-onset focal dystonia. Specifically, we were interested in measures of sensory inhibition and performed, according, an extensive electrophysiological battery, as detailed below.

5.2 Methods

5.2.1 Participants

A total of 19 consecutive patients with a diagnosis of idiopathic isolated CD according to current criteria (Albanese et al., 2013) were prospectively recruited from those attending the outpatient clinics at the National Hospital for Neurology and Neurosurgery, London, UK. Patients were assessed at least 3 months after their last botulinum toxin injection, and their disease severity was assessed with the TWSTRS. Nineteen healthy volunteers with similar age and gender distribution and no reported family history for any neurological disorders, including dystonia, served as HC. Additional exclusion criteria for both patients and HC were (1) no history of other neurological or psychiatric diseases, (2) no history of medications acting on the CNS, and (3) no symptoms or signs suggestive of a peripheral neuropathy.

5.2.2 Procedure

In all subjects STDT was collected on the right index finger and all underwent an extensive neurophysiological battery including measures of sensory excitability and inhibition (SSEP, SSEP recovery cycle, HFO, SSEP lateral inhibition; cf. Chapter 3) at both cortical and subcortical levels.

5.2.3 Statistical analysis

Given that many of the gathered variables did not distribute normally, nonparametric analyses, including the Mann–Whitney U-test and the Kruskal–Wallis test, along with the χ-2 test were used, as appropriate, to check differences between the patients and HC. Correlations between the variables were evaluated with the Spearman rank correlation coefficient. Finally, a logistic regression analysis with forward stepping (likelihood ratio method) was used to evaluate the major contributors to the variation in STDT. Thus, STDT (dependent variable) was dichotomized to the median value in HC. All significant variables in the bivariate analysis as well as those that have been demonstrated to influence the outcome (e.g., age, dystonia) were included in the model with forward stepping until adding any further single variable did not improve the model.

5.3 Results

Table 5.1 summarizes the demographic, clinical, behavioural and electrophysiological findings in patients and HC.

	HC	Patients	p
Age	57.6±14.5	62.6±9.2	0.21
Gender (F/M)	7/12	10/9	0.32
Handeness (R/L)	19/0	19/0	-
Disease duration (years)	-	9.42±4.7	-
Disease severity (TWSTRS score)	-	26.5±3.7	-
STDT (ms): mean values range	 80.1±29.9 23.3-116.7	 100.1±25.3 53.3-146.7	 **0.03**
SSEP latency (ms): - N20 thumb - N20 index - P14 thumb - P14 index	 22.35±0.9 22.96±0.9 16.33±0.6 16.48±0.6	 22.71±1.1 22.49±1.1 16.41±0.6 16.53±0.6	 0.16 0.12 0.54 0.44
SSEP amplitude (μV): - P14 thumb	 0.43±0.1	 0.41±0.1	 0.27

- P14 index	0.55±0.1	0.49±0.1	0.26
- N20 thumb	0.71±0.1	0.69±0.1	0.31
- N20 index	0.68±0.1	0.65±0.1	0.54
SSEP P14 recovery cycle amplitude ratio (μV):			
- R5	0.54±0.1	0.63±0.1	**0.02**
- R20	0.75±0.1	0.79±0.1	0.17
- R40	0.91±0.1	0.95±0.1	**0.02**
SSEP N20 recovery cycle amplitude ratio (μV):			
- R5	0.53±0.16	0.68±0.27	**<0.01**
- R20	0.71±0.13	0.82±0.89	**<0.01**
- R40	0.91±0.05	0.96±0.03	**<0.01**
Sensory lateral inhibition amplitude ratio (μV):			
- P14 sum	0.91±0.2	0.89±0.2	0.45
- P14 double pair	0.69±0.1	0.84±0.2	**<0.01**
- P14 SIR	0.72±0.1	1.03±0.1	**<0.01**
- N20 sum	1.31+0.2	1.29+0.3	0.18
- N20 double pair	0.89±0.2	1.27±0.2	**<0.01**
- N20 SIR	0.73±0.1	1.09±0.1	**<0.01**
HFOs area amplitude (μV):			
- early	3.9±1.1	3.2±0.9	**0.02**
- late	3.9±1.5	3.2±0.9	0.09

Table 5.1 Summary of the demographic, clinical, behavioural and electrophysiological features in HC and patients. Data are expressed as mean±SD, unless otherwise specified. Significant values are indicated in bold.

In summary, STDT was significantly higher in patients than HC (100.1±25.3 ms vs 80.1±29.9 ms, respectively, $p < 0.03$). Many of the sensory electrophysiological measures of temporal inhibition were also abnormal in the patients. When compared with the HC, paired-pulse SSEP data showed reduced P14 suppression at ISIs of 5 and 40 milliseconds, whereas N20 suppression was reduced at all ISIs (i.e., 5, 20, and 40 milliseconds). The e-HFO area was smaller in patients than HC, whereas there was a non-significant tendency for l-HFO to be smaller in patients.

Electrophysiological measures of spatial inhibition following simultaneous stimulation from the thumb and index finger were also reduced. In patients, the P14 and N20 SSEP responses elicited by dual stimulation were larger than the expected sum of each alone, whereas this was not the case in HC (Fig. 5.1).

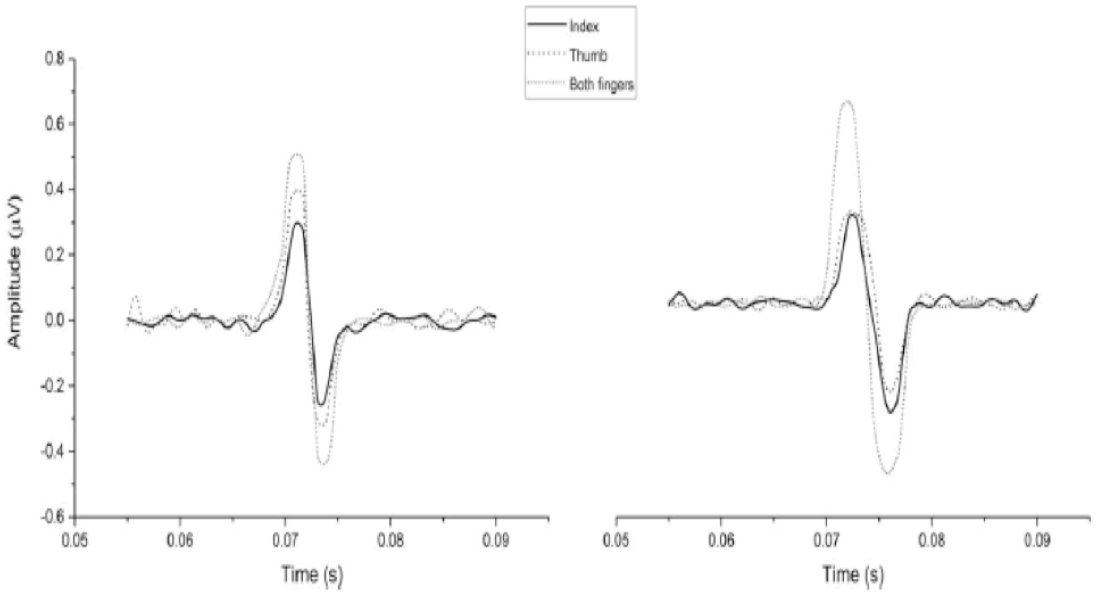

Figure 5.1 Example of paired-pulsed SSEP in one representative healthy subject (left panel) and patient (right panel), showing less suppression (i.e., lateral inhibition) in the patient when the thumb and index finger were stimulated at the same time, while SSEP from individual fingers are similar.

In both HC and patients, there was a strong correlation between STDT and N20 suppression at an ISI of 5 milliseconds (Spearman's rho 0.73, $p<0.01$ and 0.80, $p<0.01$, HC and patients, respectively) and between STDT and l-HFO area (Spearman's rho 20.73, $p<0.01$ and 20.78, $p<0.01$, HC and patients, respectively). In addition, N20 suppression at an ISI of 5 milliseconds was correlated with l-HFO area (Spearman's rho 20.84 and 20.81, HC and patients, respectively, both $p<0.01$; Fig. 4.2). There were no significant correlations with any of the other physiological measures.

There were also no correlations between STDT and disease duration or severity in the patient group as assessed by the TWSTRS.

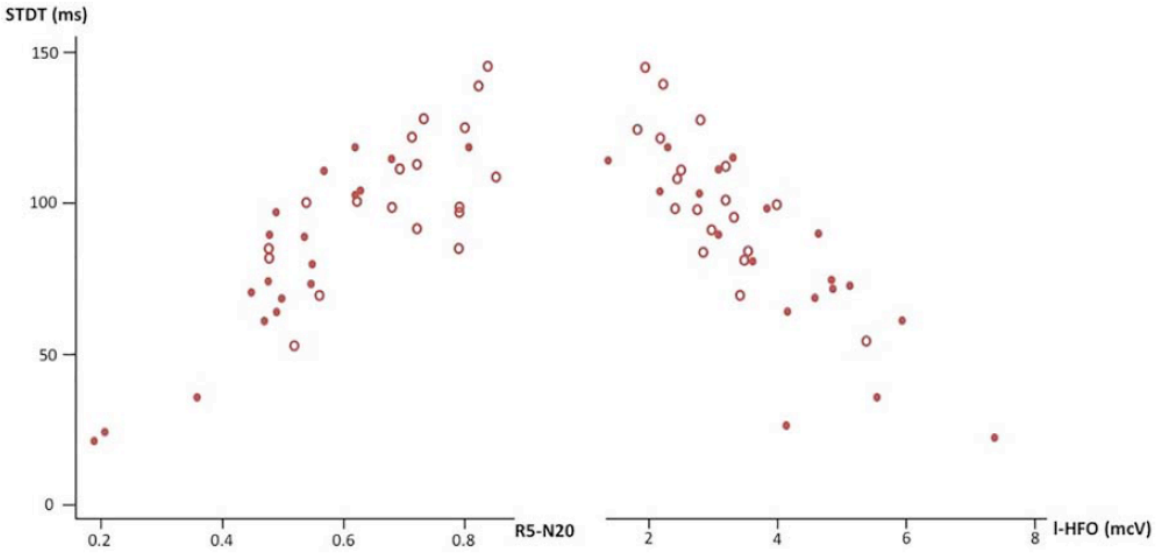

Figure 5.2 Correlations between STDT and suppression of the N20 at 5 ms ISI (left panel) and l-HFO (right panel) in healthy subjects (red dots) and patients with CD (empty dots).

Finally, the logistic regression model showed that reduced N20 suppression at an ISI of 5 milliseconds (β coefficient 67.33; $p<0.01$), smaller l-HFO area (β coefficient 211.05; $p<0.01$), and (dystonia) group (β coefficient 9.62; $p<0.05$), were independently associated with higher STDT, explaining a variance of 64% ($R^2=64.5$). The Hosmer–Lemeshow goodness-of-fit test supported our regression model as being valid.

5.4 Discussion

In line with previous studies (Avanzino et al., 2015; Bradley et al., 2010; Bradley et al., 2009; Fiorio et al., 2007; Fiorio et al., 2008; Tinazzi et al.,

1999), we found higher STDT in patients than in HC. The fact that we observed abnormal STDT in non-dystonic body regions together with the lack of correlation between STDT and dystonia severity further confirms the notion that higher STDT in patients is not merely a consequence of overt manifestations of dystonia (Avanzino et al., 2015).

Our mean STDT values in both HC and patients were slightly higher than reported in some previous studies. Several factors could contribute to this, including the older age of our cohorts as well as the different procedures that have been used in different studies (i.e., ascending or descending method, use of different intensity for the stimuli, assessment of uni- vs multimodal TDT, etc.). In line with this, Giersch and colleagues demonstrated that TDTs obtained using different protocols/equipment are only comparable within each individual experimental paradigm (Giersch et al., 2009).

As mentioned earlier, Tamura et al. found that patients with FHD had reduced suppression of the P27 component of the SSEP following pairs of stimuli at 5 milliseconds, but not at other ISIs (Tamura et al., 2008). The present results confirm that paired-pulse suppression of the N20 at the ISI of 5 milliseconds (that is equivalent to the P27 of Tamura et al. because we measured the same peak-to-peak N20-P27 SSEP component) was reduced in patients when compared with the control group. We also observed a reduced suppression at the ISI of 20 and 40 milliseconds, which were not evident in the previous study (Tamura et al., 2008). This may be a result of the fact that our SSEPs were elicited by stimulation of the digital nerves of the index finger rather than the median nerve at the wrist. The smaller SSEPs from digital stimulation may be in fact more sensitive to changes in cortical inhibition. Moreover, reduced suppression at ISI of 20 and 40 milliseconds has been reported in patients with segmental and generalized dystonia.

SSEP suppression of the N20 at short intervals (ISI of 5 milliseconds) is thought to be primarily of cortical origin, whereas suppression at longer ISIs (i.e., ISI of 20 and 40 milliseconds) is mediated by inhibitory postsynaptic interneurons within the dorsal column nuclei and the thalamus (ventral postero-lateral nucleus) (Araki et al., 1997; Emori et al., 1991; Lueders, Lesser, Hahn, Little, & Klem, 1983; Lueders, Lesser, Hahn, Dinner, & Klem, 1983; Meyer-Hardting, Wiederholt, & Budnick, 1983). The evidence that abnormal processing of paired-pulse SSEP occurs in dystonia also at the subcortical levels is further supported by the fact that we found reduced suppression of the SSEP P14 component. In fact, its suppression reflects inhibitory activity within the dorsal column-lemniscus medialis (Lueders, Lesser, Hahn, Little, et al., 1983).

We found reduced e-HFO area in patients and a similar non-significant trend for l-HFO. HFO are low-amplitude, high-frequency wavelets superimposed on the N20 wave, with their early component suggested to represent activity from thalamo-cortical fibers projecting mainly to area 3b and 1 within S1, whereas the late component represents activity of S1 inhibitory interneurons (Murakami, Sakuma, & Nakashima, 2008; Murakami, Sakuma, Nomura, Nakashima, et al., 2008; Murakami, Sakuma, Nomura, Uemura, et al., 2008; Ozaki & Hashimoto, 2011). In line with our results, 1 previous study in patients with CD found HFO to be reduced (Inoue et al., 2004).

As to lateral inhibition, we found a significant difference between dystonic patients and HC, which was not the case in a previous study (Tinazzi et al., 2000). Given that lateral inhibition is mediated by intra-cortical connections within a limited range (Helmstaedter, Sakmann, & Feldmeyer, 2009) and that contiguous fingers are represented adjacently in S1 (Kolasinski et al., 2016), it is likely that inhibition is stronger when tested in adjacent fingers. Thus, the significant difference we found between the

2 groups might be accounted for by the fact that we tested lateral inhibition stimulating the thumb and index finger rather than 2 non-contiguous fingers. In addition, the difference in the sample size (19 vs 7 patients) might also explain the different result.

Overall, differences in SSEP between patients and controls were observed in both temporal and spatial domains, suggesting a widespread deficit of sensory processing. However, the latter finding (e.g., impaired sensory lateral inhibition) did not correlate with abnormal STDT, suggesting that increased STDT in dystonia is not merely owing to abnormal cortical activity, but is the result of specific abnormalities within circuits processing the temporal aspects of afferent inputs (e.g. SSEP recovery cycle and HFO).

In fact, only some of these measures, namely the suppression of the N20 at 5-millisecond ISI and the l-HFO, individually correlated with STDT and were independently associated with STDT in the logistic regression model. These measures likely rely on local inhibition within S1 (Murakami, Sakuma, Nomura, Nakashima, et al., 2008; Tamura et al., 2008; Tamura et al., 2009), and therefore these inhibitory intra-cortical circuits might act to sharpen the distinction between the first and the second afferent inputs in STDT (Rocchi, Casula, et al., 2016).

The regression analysis also indicated that a separate factor "dystonia group" was also predictive of higher STDT. This suggests that there is one or more additional factors beyond our measures of cortical somatosensory inhibition that contributes to higher STDT in patients. This is somewhat supported by the fact that the regression model only explained 65% of the variance, indicating that other factors contribute to the behavioural performance. Previous imaging studies exploring abnormal STDT in dystonia have found somewhat contradictory results, reporting structural and functional abnormalities either at subcortical (putamen) (Bradley et

al., 2009; Kimmich et al., 2014) and cortical (middle frontal, precentral, and postcentral giri) levels (Kimmich et al., 2014; Termsarasab et al., 2016), thus leaving the question of which are additional contributors to abnormal STDT in dystonia open.

We cannot conclude with any certainty whether the reduced inhibition in S1 developed secondarily to pathology in the basal ganglia or occurred independently. Either way, these results are consistent with the concept of dystonia being a network disorder involving different nodes within the CNS and with higher STDT being largely, but not completely, explained by reduced cortical inhibition (Rocchi, Casula, et al., 2016; Tamura et al., 2008). Abnormal activity within the basal ganglia (Peller et al., 2006; Schneider et al., 2010) might play an additional role in modulating STDT.

While performing the neurophysiological investigations, we took great care to ensure that both patients and HC were seated comfortably and quietly to avoid the occurrence of involuntary movements. Obviously, we cannot entirely exclude that intermittent head movements in dystonic patients might have played a minor role in reducing inhibition within the sensory system because it is well known that movement gates sensory access to cortex (Murase et al., 2000). Nonetheless, this possible limitation would not account in our view for the overall observed results.

In conclusion, in this work we have demonstrated that abnormal STDT in CD is crucially dependent on inhibitory mechanisms acting within S1. These might hence theoretically represent a therapeutic target to reverse this behavioural deficit in patients with dystonia.

Chapter 6:
High frequency repetitive sensory stimulation reversibly improves STDT in healthy subjects

The work presented in this chapter was originally published in the form of a research article: Erro R, Rocchi L, Antelmi E, et al. High frequency repetitive sensory stimulation improves temporal discrimination in healthy subjects. Clin Neurophysiol. 2016;127:817-20.

6.1 Introduction

As we have seen earlier (cf. Chapter 2), Dinse and colleagues have shown that HF-RSS can improve sensory perception in the stimulated area in humans (Dinse et al., 2006). Namely, they have demonstrated that perceptual performance, in terms of TPDT, improved after such a NIBS protocol (Dinse et al., 2006). This result appeared, at first sight, difficult to explain based on the evidence that HF-RSS increases the representational area of the stimulated digit in sensory cortex in humans (Pleger et al., 2003) and increases the receptive field size of individual cortical neurons in animal experiments (Godde et al., 1996). In fact, as the authors pointed out, an initial expectation might be that larger receptive fields would reduce perceptual acuity. However, perceptual ability does not necessarily relate to the receptive field size of individual neurons, but instead reflects the sum total of information present in the discharge of many neurons (Dinse et al., 2006; Godde et al., 1996; Godde et al., 2000). Thus, increasing numbers of neurons responsive to inputs from an area of skin that have overlapping and slightly different receptive fields, can code acuity with greater precision than any single neuron alone.

Theoretically, the same argument can be applied to temporal acuity: The summed activity of greater numbers of neurons responding to an input

may be capable of higher temporal resolution than any one neuron alone. However, this has never been tested formally in the context of HF-RSS. In fact, Godde et al. found that the response duration of sensory neurons increased after conditioning in rats (Godde et al., 1996), which could potentially reduce temporal resolution.

Being most interested in STDT as it has been largely reported to be abnormal and to represent an endophenotypic marker of dystonia (cf. chapter 1), we aimed in the current experiments to examine the consequences for temporal somatosensory perception of HF-RSS from a digit in healthy young volunteers. Moreover, since Dinse et al. had shown that the effect of HF-RSS on spatial discrimination was larger in the elderly than in young participants (Dinse et al., 2006), in a second experiment we asked whether the effects of HF-RSS were any different in a group of healthy elderly.

6.2 Methods

6.2.1 Participants

Twelve healthy young subjects (seven females; aged 28–32 years – Group A) and 10 healthy right-handed elderly subjects (4 females; aged 50–76 years – Group B) participated in the current set of experiments. Participants had no history of any neuropsychiatric disorders, neurosurgery, or metal or electronic implants and were not on drugs active at CNS level at the time of the experiments.

6.2.2 Procedure

HF-RSS was applied on the right index finger. In experiment 1 (i.e. involving group A), STDT was collected before (T0), soon after (T1), 2,5 h (T2) and 24 h (T3) after HF-RSS on the right index finger (i.e., the target finger) and on the right thumb and left index finger (i.e., both considered as control fingers). In experiment 2, SDTD was only collected before and

soon after HF-RSS in group B (i.e., elderly group) to evaluate any age-related differences between groups. As in experiment 1, SDTD was collected on the right index finger and thumb and the left index finger.

6.2.3 Statistical analysis

To understand which factor(s) could influence the outcomes we fitted three mixed linear models for repeated measures. Two separate models were run on each of the two groups considering ‘‘time’’ and ‘‘finger’’ as factors, and the interaction term between the two variables, including for the group of young subjects the complete set of four outcome measurements. We then fitted a general model considering ‘‘group’’, ‘‘time’’ and ‘‘finger’’ as covariates, as well as the interaction term between time and finger and the interaction term between time and group. To test the goodness of fit the interclass correlation was checked for the three models.

Finally, we performed a post hoc pairwise simple effects test when an interaction effect was found to be significant. All values are expressed as mean ± standard deviation. P values, F test and degrees of freedoms (df) are reported. Results were considered statistically significant for $p<0.05$.

6.3 Results

Baseline (pre-conditioning) values of STDT were similar for the three examined fingers in both groups ($p>0.05$).

The mixed linear model for the young subjects showed a significant main effect of ‘‘time’’ ($p < 0.000$, $F = 33.94$, $df = 3$) and an interaction between “time’’ and ‘‘finger’’ ($p < 0.000$, $F = 30.71$, $df = 6$). We therefore explored the effect of time separately in each finger and found that it significantly influenced STDT only in the stimulated finger ($p < 0.000$, $F = 94.19$, $df = 3$, Fig. 6.1, top panel; STDT at T0:77.1 ± 16.5 ms vs. T1: 45.6 ± 19.1 ms, T2: 57.2 ± 20.8 ms, and T3: 76.4 ± 16.1 ms; max percentage reduction occurring at T1: about 41% of baseline STDT values). STDT values were

unchanged in both right thumb and left index ($p > 0.05$, right thumb STDT at T0: 74.3 ± 17.6 ms vs. T1: 75.2 ± 18.7 ms, T2: 77.2 ± 18.2 ms, and T3: 77.3 ± 18.4 ms; left index STDT at T0: 75.7 ± 18.7 ms vs. T1: 75.5 ± 21.2 ms, T2: 74.9 ± 15.4 ms, and T3: 76.9 ± 17.1 ms)

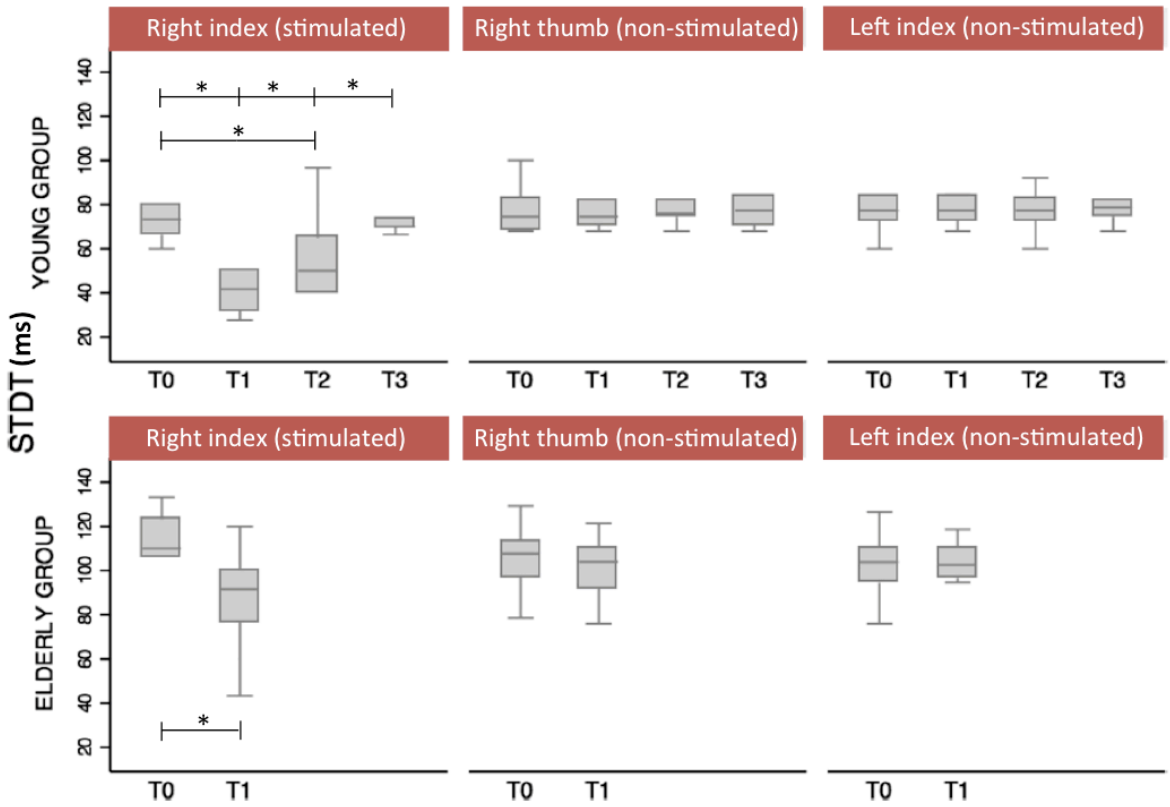

Figure 6.1 Box whisker plot showing the distribution of STDT values on the stimulated finger (right index) and on the non-stimulated fingers (right thumb and left index) before (T0), 5 min (T1), 2.5 h (T2) and 24 h (T3) after the stimulation protocol in the young (top panel) and in the elderly group (bottom panel). Vertical bars represent SD. Stars indicate statistical significance ($p<0.05$).

In the elderly group, baseline STDT values on the stimulated finger were significantly higher than in the young group (106.2 ± 23.9 ms vs. 77.1 ± 16.5 ms, $p < 0.01$). The mixed linear model showed a significant effect of

‘‘time’’ ($p < 0.001$, $F = 6.24$, $df = 1$) and an interaction between ‘‘time’’ and ‘‘finger’’ ($p < 0.001$, $F = 7.07$, $df = 2$). We therefore examined the effect of time separately on each finger. There was a significant effect only for the stimulated finger (Fig. 6.1, bottom panel, $p < 0.001$, $F = 19.42$, $df = 1$, STDT at T0: 106.2 ± 23.9 ms vs. T1: 86.33 ± 23.9 ms; percentage reduction: about 19%), with no change in the non-stimulated fingers ($p > 0.05$, right thumb STDT at T0: 103.6 ± 22.5 ms vs. T1: 100.7 ± 18.3 ms; left index STDT at T0: 99.3 ± 22.4 ms vs. T1: 102.6 ± 15.1 ms).

The general model showed that the interaction term between time and group was significant (e.g., group A: $p < 0.01$, $F = 29.99$, $df = 1$; group B: $p<0.01$, $F = 10.17$, $df = 1$). In fact, the magnitude of improvement was significantly larger in the young than the elderly subjects (about 41% and about 19% of baseline STDT values, respectively, $p < 0.01$, $F = 13.41$, $df = 1$,), despite same duration and intensity of stimulation (i.e., 300% of the sensory threshold).

6.4 Discussion

In the current set of experiments, we have demonstrated that 45 minutes of an unattended HF-RSS protocol improves temporal discrimination abilities in healthy volunteers. The effect is reversible, with STDT returning to the baseline values within 24 h, and is larger in young than in elderly individuals. Moreover, it is specific for the stimulated finger (right index), indicating that it is not due to practice on the task.

In their extensive work on spatial discrimination, Dinse and colleagues speculated that repeated “co-activation” of sensory inputs increases the area of somatosensory cortex responsive to that input. Even though co-activation enlarges the spatial receptive field of individual neurons, the fact that more neurons respond to the input allows the perceptual system to extract more precise spatial information from a population of

overlapping and slightly different RFs (Dinse, 2006; Dinse, Ragert, Pleger, Schwenkreis, & Tegenthoff, 2003; Godde et al., 2002; Godde et al., 1996; Godde et al., 2000; Pleger et al., 2001; Pleger et al., 2003; Pleger et al., 2006; Schlieper & Dinse, 2012). It is therefore possible that a similar mechanism explains the increased temporal resolution that we observed in the present experiments. This would also explain why the effects are only observed onto the stimulated site, since the effects at a cortical level are somatotopically limited to co-activated inputs at the site of stimulation.

Another possible explanation is that HF-RSS changes the properties of inhibitory neurons in sensory cortex. One class of these is excited monosynaptically by thalamo-cortical inputs and exerts feed-forward inhibition on the post-synaptic cortical neurons in S1 (Beierlein, Gibson, & Connors, 2003; Hestrin & Galarreta, 2005). By quickly terminating any initial excitation produced by thalamic inputs, these neurons could sharpen the temporal features of sensory inputs. If repeated activation of these neurons during HF-RSS increased their effectiveness, then it could potentially increase temporal discrimination. This would also be consistent with a study by Tamura et al. (Tamura et al., 2008), which showed that increased STDT values are associated with altered somatosensory intra-cortical inhibition.

As reported by others (Ramos et al., 2016), our elderly volunteers had a higher STDT than the younger group. Even though HF-RSS improved STDT in the elderly, their mean values still lay above those of younger individuals. Previous work on spatial discrimination has shown that higher intensity HF-RSS can lead to a greater improvement in perception (Schlieper & Dinse, 2012). However, this approach could not be used to increase the effect in our elderly group since we used the maximum tolerated intensity of stimulation. Another possibility is that a further

improvement could be obtained by simply increasing the duration of stimulation (i.e., by increasing the total number of stimuli each session or perhaps applying multiple sessions on consecutive days) but this has yet to be investigated.

There is one aspect of our data that contrasts with the previous work of Dinse and colleagues (Dinse et al., 2006). They found that the improvement in spatial discrimination in the elderly was several-fold stronger than in young subjects (Dinse et al., 2006), whereas we found the opposite for temporal discrimination. It is not clear why this should be if the explanation for both phenomena depends on increasing the number of responsive neurons in the cortex. On the one hand, it should be stressed that the neural mechanisms that decode spatial and temporal discrimination from the raw input signal to cortex are likely to be different and, hence, might respond differently to such a stimulation protocol, despite the overall outcomes being similar. On the other hand, our results nicely fit with the general view that neuronal plasticity tends to become much less profound in the aged brain (Hubener & Bonhoeffer, 2014). In this regard, it should also be acknowledged that the elderly participants were tested at only two time points and therefore definitive conclusions cannot be drawn on whether the time course of STDT improvement following the HF-RSS in the elderly parallels that observed in the group of young subjects. Further studies are needed to answer this question.

Our results show that the HF-RSS protocol reversibly improves temporal discrimination in both young and elderly healthy subjects, although the magnitude of the effect was larger in the young group. It seems likely that some of these effects are caused by plastic changes in S1.

Chapter 7:
High frequency sensory stimulation increases sensorimotor inhibition in healthy subjects

The work presented in this chapter was originally published in the form of a research article: Rocchi L, Erro R*, Antelmi E, et al. High frequency somatosensory stimulation increases sensorimotor inhibition and leads to perceptual improvement in healthy subjects. Clin Neurophysiol. 2017; 128:1015-1025. [*joint first author]*

7.1 Introduction

In the previous chapter, we have seen that a 45-minute session of HF-RSS could reversibly improve STDT on the stimulated finger in healthy subjects. These results mirrored those from other authors showing an improvement in a spatial discrimination task on the stimulated area (Dinse et al., 2006; Godde et al., 2002; Godde et al., 1996; Godde et al., 2000; Pleger et al., 2001; Pleger et al., 2003). In these initial works, however, the perceptual improvement was accompanied by an increase of the RF of the stimulated area in animal models (Godde et al., 1996), and by an enlargement of cortical representational areas in humans (Pleger et al., 2003). The latter findings would appear counter-intuitive since enlarged (i.e., less-defined) receptive fields should theoretically lead to less-accurate tactile acuity. The authors put forward a partial proposal to account for this discrepancy, suggesting that (spatial) discrimination abilities do not necessarily relate to the RF size of individual neurons, but instead reflects the sum total of information present in the discharge of many neurons (Dinse et al., 2006; Godde et al., 1996; Godde et al., 2000). More neurons responsive to inputs from an area of skin with overlapping and slightly different RF would therefore code spatial representation with

greater precision than any single neuron alone. While this is conceivable, such an explanation also implies that there should be a predicted RF size where overlap (and hence tactile acuity) is maximal. In other words, the increase in size of RF does not necessarily imply, by itself, an improvement in tactile acuity. An increased load of inhibition to maintain RF size close to the point where accuracy is maximal should be observed. Hence, it was our expectation that an improvement of tactile acuity in the spatial domain should have been driven both by larger and partially overlapping RF as well as by increased effectiveness of inhibitory connections between adjacent fields. The latter two complementary effects would explain the behavioural improvement better than either alone.

Translating this hypothesis into the temporal domain, the observed improvement of STDT following a 45-minute session of HF-RSS (cf. Chapter 5) could be explained by the combination of two effects. More accurate temporal discrimination might result from engagement of larger numbers of neurones involved in temporal processing as well as increased inhibition that, quickly terminating any initial excitation produced by consecutive stimuli, could sharpen the temporal features of sensory inputs (Rocchi, Casula, et al., 2016).

We had previously linked STDT with the efficacy of inhibitory circuitry within S1 (cf. Chapter 5). Therefore, our hypothesis was that HF-RSS could potentiate intra-cortical inhibitory circuitry within S1 and, thus, lead to decreased STDT (i.e., better performance).

As detailed in the next paragraph, we performed an extensive electrophysiological battery tapping not only measures of sensory excitability/inhibition, but also of motor excitability/inhibition. This was because a previous study had shown that HF-RSS could change the EEG mu rhythm not only over the sensory areas, but also over the motor areas (Freyer et al., 2012). We were hence also interested in addressing the

question of whether the physiological effects of HF-RSS in the sensory domain could be somewhat transferred to motor cortical areas.

7.2 Methods

7.2.1 Participants

Fifteen healthy subjects (11 male, 4 female, age 54.53±16.38), all right handed (Oldfield, 1971), were enrolled in the study. Participants had no history of any diseases related to the central or peripheral nervous system; they did not have metal or electronic implants and were not taking drugs active on the CNS.

7.2.2 Procedure

HF-RSS was applied on the right index finger. The STDT was collected before (T0) and after HF-RSS (T1) in the right index finger (i.e. target finger) and right thumb and left index finger (i.e., control fingers), as in the previous experiment (cf. Chapter 5). At both T0 and T1, all subjects further performed an extensive electrophysiological battery tapping measures of sensory excitability and inhibition (SSEP, SSEP recovery cycle, HFOs) and motor excitability and inhibition (SICI and ICF), as detailed in Chapter 3. T0 and T1 electrophysiological measurements (i.e., SSEP vs TMS) were counterbalanced across subjects.

7.2.3 Statistical analysis

Since Shapiro-Wilks' test was non-significant ($p>0.05$) for the gathered variables, parametric tests were performed with Greenhouse-Geisser correction to correct for non-sphericity, when necessary (i.e. Mauchly's test < 0.05). A three-way repeated measures ANOVA with "time" (T0, T1), "side" (right, left) and "finger" (thumb, index finger) as factors of analysis was performed to evaluate the effect of HF-RSS on STDT. Four different dependent T-tests were used to evaluate the effect of HF-RSS on the latency and of N20 and P14, each recorded from the right thumb and right

index finger. Four different dependent T-tests were used to evaluate the effect of HF-RSS on the amplitude of N20 and P14, each recorded from the right thumb and right index finger. Two individual two-way repeated-measures ANOVA with “time” (T0, T1) and “ISI” (R5, R20, R40) as factors of analysis were performed to investigate the effect of HF-RSS on N20 and P14 recovery cycle. Two dependent t-tests were used to investigate possible effects of HF-RSS on e-HFO and l-HFO. Pearson's correlation coefficient was used to investigate possible correlations between baseline STDT measured on the right index finger, e-HFO area, l-HFO, and SSEP recovery cycle. A three-way repeated measures ANOVA with “time” (T0, T1), “muscle” (FDI, APB, ADM) and “condition” (test pulse, SICI 70%, SICI 80%, SICI 90%, ICF) as factors of analysis was used to disclose possible effects of HF-RSS on SICI, and ICF. Bonferroni post-hoc test was used for all post-hoc analyses; p values < 0.05 were considered significant.

7.3 Results

7.3.1 Somatosensory temporal discrimination threshold

Overall, the same current intensity was used for STDT while testing different fingers at different time points. As reported previously (Erro et al., 2015), HF-RSS improved STDT in a spatially specific manner.

The ANOVA showed a non-significant main effect of “time”, “side” and “finger”, and non-significant interactions between these factors (all $p > 0.05$). However, the three-way ANOVA on STDT values showed a significant main effect of "time" [$F(1,14) = 14.624$; $p = 0.002$], a non-significant effect of "side" [$F(1,14) = 1.104$; $p = 0.311$], and "finger" [$F(1,14) = 2.085$; $p = 0.171$], significant interactions of "time×side" [$F(1,14) = 35.681$; $p < 0.001$] and "time×finger" [$F(1,14) = 8.172$; $p = 0.013$], a non-significant interaction of "side×finger" [$F(1,14) = 0.396$; $p = 0.539$] and a significant interaction of "time×side×finger" [$F(1,14) = 8.823$; $p = 0.01$].

Post-hoc analyses showed that STDT significantly decreased in the right index finger from T0 to T1 (87.62±36.01 vs. 68.60±37.13; $p < 0.001$), while it remained unchanged in the other fingers (Fig. 7.1).

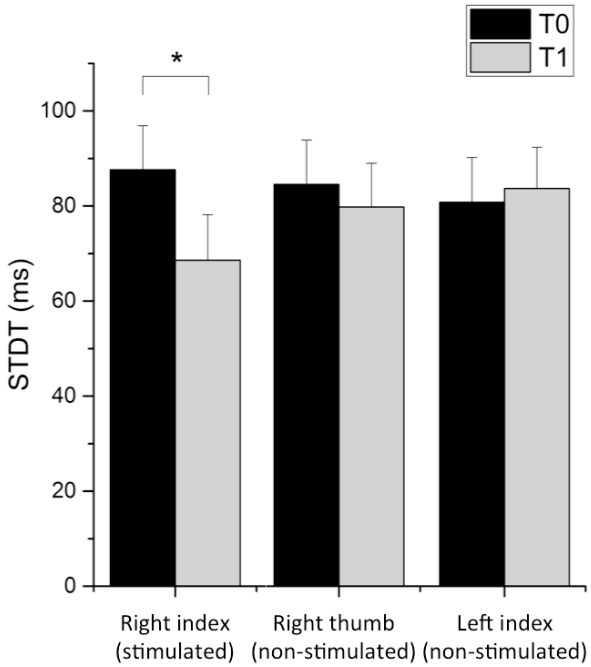

Figure 7.1 STDT values on the stimulated finger (right index) and on the non-stimulated fingers (right thumb and left index) before (T0) and after (T1) the stimulation protocol. Vertical bars represent SD. Stars indicate statistical significance ($p<0.05$).

7.3.2 N20 and P14 latency and amplitude

HF-RSS had no effect on the latency of these early SEP components, but significantly increased their amplitude.

The t-tests run to assess a possible effect of HF-RSS on the latency of N20 and P14 did not show any significant effects (all $p>0.05$). By contrast, HF-RSS significantly increased the amplitude of N20 [$t(15) = -11.386$; $p < 0.001$] and P14 [$t(15) = -10.862$; $p < 0.001$] obtained by stimulation of the

right index finger, while no changes were observed in N20 and P14 recorded while stimulating the right thumb (all p>0.05).

7.3.3 SSEP recovery cycle and HFO

HF-RSS increased the amount of inhibition produced by the first stimulus of the pair on both the N20 and P14 components. Thus, the recovery cycle was suppressed at all three intervals tested.

The two-way ANOVA performed to disclose possible effects of HF-RSS on the N20 recovery cycle showed a significant main effect of "time" [$F(1,14) = 70.02$; $p < 0.001$] and "ISI" [$F(1.479,17.234) = 38.816$; $p < 0.001$] and a significant interaction of "time×ISI" [$F(1.949,27.282) = 4.014$; $p = 0.031$]. Post-hoc comparisons showed that inhibition increased from T0 and T1, and this was true for R5 (0.53 ± 0.19 vs. 0.37 ± 0.16; $p < 0.001$), R20 (0.72 ± 0.11 vs. 0.52 ± 0.12; $p < 0.001$) and R40 (0.92 ± 0.06 vs. 0.67 ± 0.14; $p < 0.001$) (fig. 4). Accordingly, HF-RSS increased inhibition also when the P14 component was considered. In this case, the two-way ANOVA showed a significant main effect of "time" [$F(1,14) = 59.48$; $p < 0.001$] and "ISI" [$F(1.540,21.561) = 136.85$; $p < 0.001$] and a significant interaction of "time×ISI" [$F(1.618,22.649) = 5.883$; $p = 0.012$]. Again, post-hoc comparisons showed an increase in inhibition from T0 and T1 for R5 (0.56 ± 0.15 vs. 0.40 ± 0.09; $p < 0.001$), R20 (0.78 ± 0.10 vs. 0.55 ± 0.08; $p < 0.001$) and R40 (0.92 ± 0.04 vs. 0.80 ± 0.06; $p < 0.001$) (Fig. 7.2). The paired t-tests showed a significant increase of e-HFO [$t(15) = -5.860$; $p < 0.001$] and l-HFO [$t(15) = -5.279$; $p < 0.001$] after HSS (fig. 7.3).

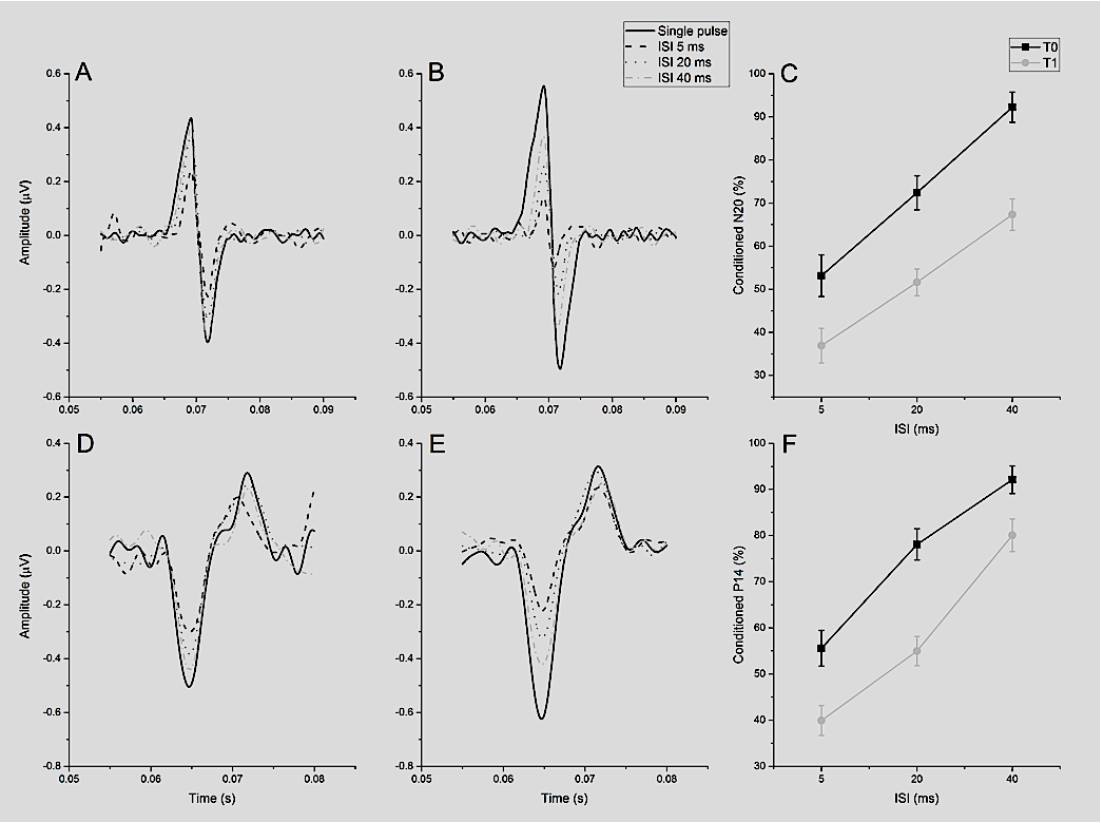

Figure 7.2 SSEP recovery cycle of N20-P25 (panels A-C) and P14 (panels D-F) components of SEP at ISIs of 5, 20 and 40 ms before (T0: panels A and D) and immediately after (T1; panels B and E) HF-RSS. HF-RSS increased the amplitude of unconditioned N20-P25 and P14 whereas it decreased the amplitude of paired pulse SSEP (i.e,, increasing the effectiveness of inhibition). For visualization purposes the raw signal was bandpassed between 20 and 500 Hz. Artefact from electric stimulus (at 0.05 s) was removed. Error bars indicate SE.

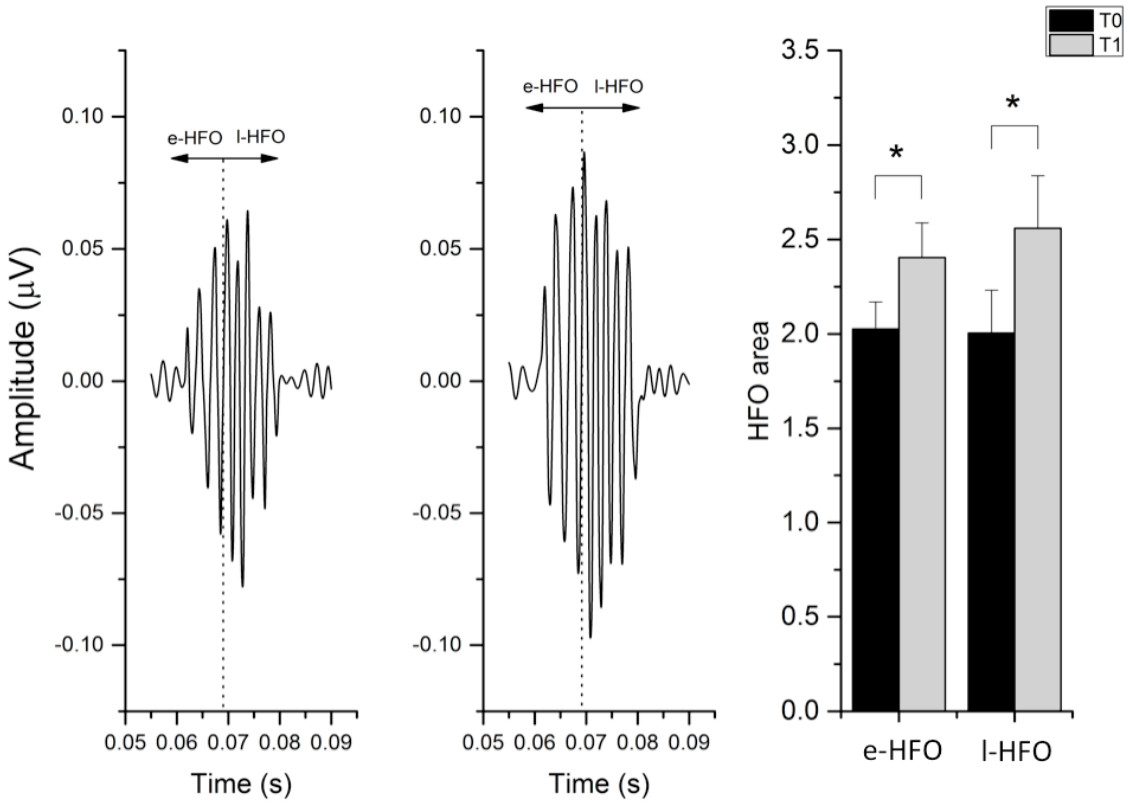

Figure 7.3 HFO before (left panel, T0) and immediately after (middle panel, T1) HF-RSS applied on the right index finger. HF-RSS induced a significant increase of both early ($p < 0.001$) and late HFO area ($p < 0.001$). HFO area in the right panel is expressed in μV2 x 10-4. Artefact from electric stimulus (at 0.05 s) was removed. Asterisks indicate statistical significance. Error bars indicate SE.

7.3.4 Correlations between behavioural and neurophysiologic measures

There was a strong correlation between changes induced by HF-RSS in physiological measures of inhibition of the N20 and l-HFO and in STDT. At baseline (i.e. T0) there were significant correlations between STDT and R5 of the N20 ($r = 0.830$; $p < 0.001$); STDT and l-HFO area ($r = -0.887$; $p < 0.001$); and R5(N20) and l-HFO area ($r = -0.690$; $p = 0.004$). In addition, the changes induced by HF-RSS in STDT were significantly correlated with the changes induced by HF-RSS on R5(N20) ($r = 0.795$; $p < 0.001$) and on l-HFO area ($r = 0.746$; $p = 0.001$) (fig. 7.4; upper panels).

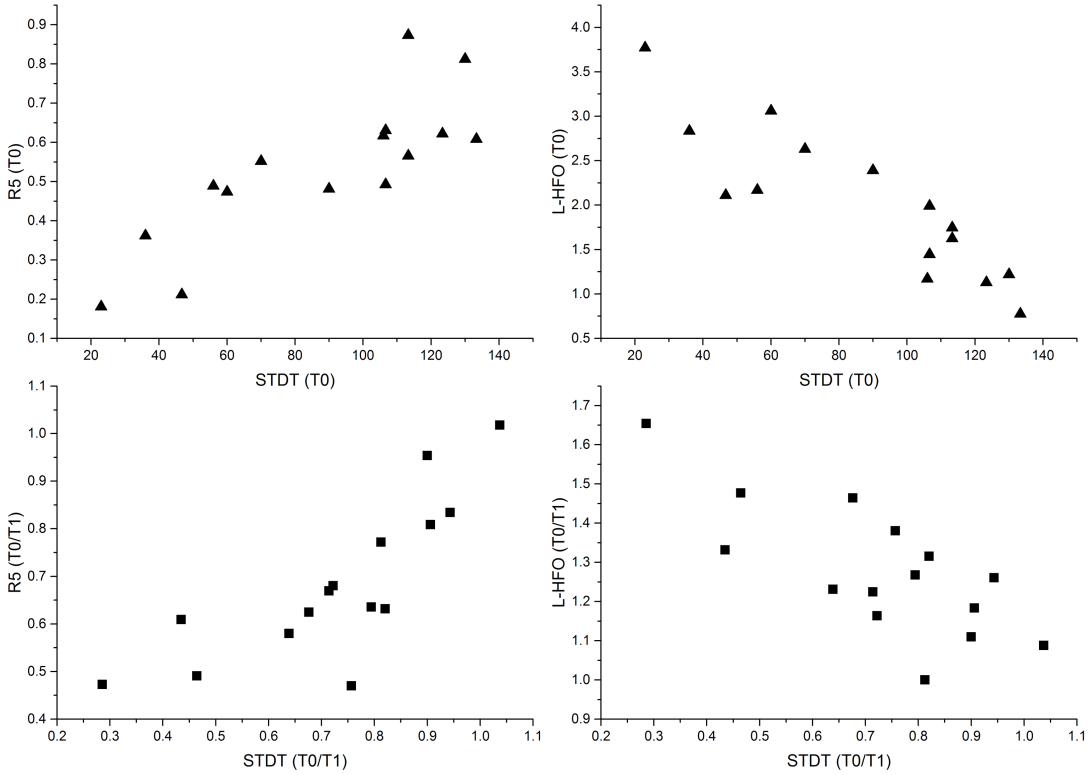

Figure 7.4 Correlations between STDT, R5-N20 and l-HFO. The upper panels show a significant correlation between values of STDT and R5 (left) and between baseline values of STDT and l-HFO (right) at baseline. There was also a significant correlation between the changes induced by HF-RSS on STDT and the changes induced, respectively, on R5-N20 (lower left panel) and on l-HFO (lower right panel).

There was also a significant correlation between changes induced in R5 and in l-HFO ($r = 0.765$; $p = 0.001$; fig. 7.4, lower panels). No correlations were found between STDT and SEP recovery at ISIs other than 5 ms, and no correlation was found between STDT and e-HFO. Notably, the changes induced by HF-RSS on R5 of the N20 and P14 were not correlated. There was no correlation between STDT and P14 recovery at any of the ISIs explored.

STDT was not correlated with TSD assessed with the JVP domes test at any time point (all p values > 0.05). Although not significant, there was a trend towards correlation between the STDT and TT at T0 ($r = 0.466$, $p = 0.08$) and T1 ($r = 0.424$, $p = 0.074$), and also the changes induced on the two variables by HF-RSS showed the same tendency ($r = 0.466$, $p = 0.08$).

7.3.5 Effect of HF-RSS on M1 inhibitory circuitry

HF-RSS produced a focal increase of SICI in APB, but had no effect on other muscles or on ICF.

The three-way ANOVA on SICI and ICF showed a non-significant main effect of "time" [$F(1,14) = 3.028$; $p = 0.104$], significant main effects of "muscle" [$F(1.907,26.702) = 33.952$; $p < 0.001$] and "condition" [$F(1.828,25.589) = 344.620$; $p < 0.001$] and significant interactions of "time×muscle" [$F(1.761,24.658) = 3.771$; $p = 0.042$], "time×condition" [$F(1.925,26.945) = 7.781$; $p = 0.002$], "muscle×condition" [$F(2.938,41.135) = 136.131$; $p < 0.001$] and "time×muscle×condition" [$F(2.885,40.391) = 5.816$; $p = 0.002$]. Post hoc analyses showed that HF-RSS had no effect on unconditioned MEP, SICI and ICF recorded on FDI and ADM (all $p > 0.05$). On APB, by contrast, while HF-RSS had no effect on test MEP and ICF (all $p > 0.05$), the amount of SICI increased from T0 to T1 (i.e. there was a decrease in the amplitude of the conditioned MEP), and this was true with

a conditioning pulse set respectively at 70% (0.76 ± 0.10 mV vs. 0.63 ± 0.06 mV; $p < 0.001$), 80% (0.53 ± 0.10 mV vs. 0.43 ± 0.09 mV; $p < 0.001$), and 90% (0.38 ± 0.07 vs. 0.29 ± 0.09; $p < 0.001$) of AMT (fig. 7.5).

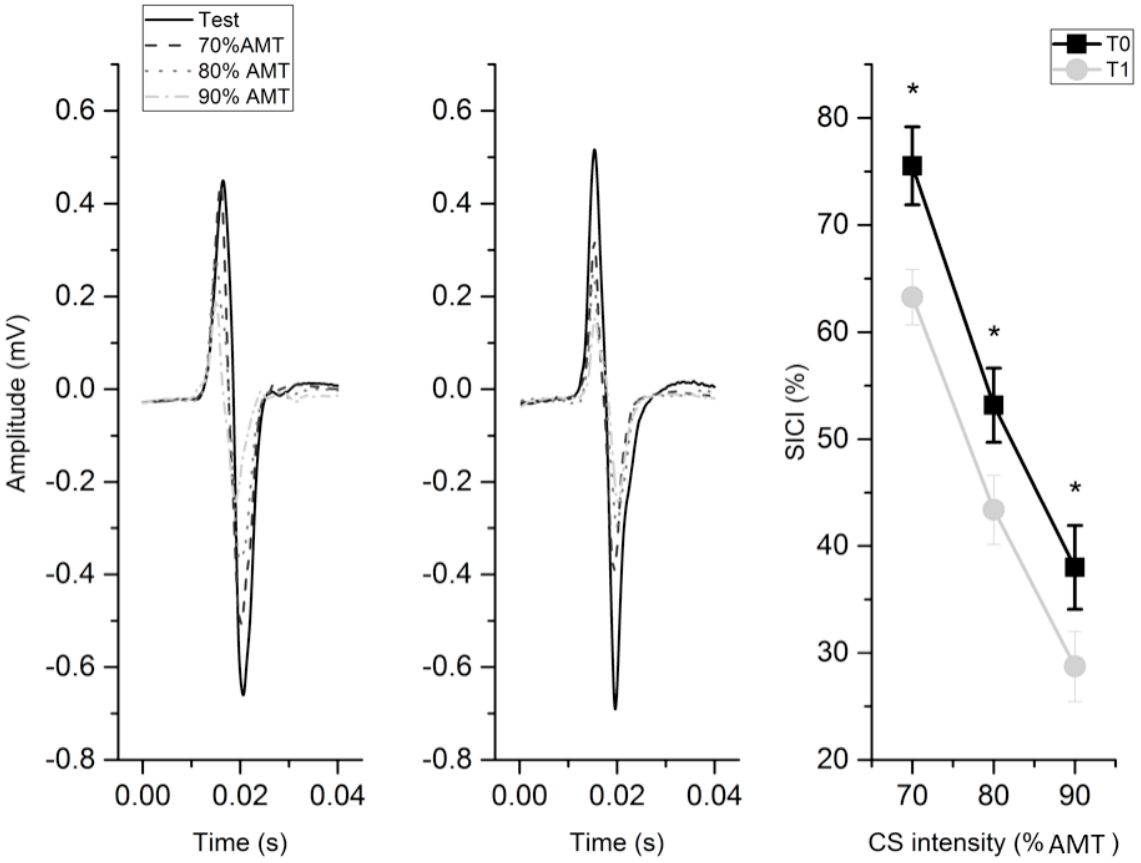

Figure 7.5 Effects induced by HF-RSS on SICI on the APB. Raw signal from the APB of a single subject before (left panel) and after (middle panel) HF-RSS using different intensities of the conditioning TMS stimulus (CS) (70, 80 and 90% of AMT). HF-RSS induced an increase in SICI irrespective of the strength of the conditioning TMS pulse (all p values <001). Right panel shows SICI averaged among all subjects. Asterisks indicate statistical significance. Vertical bars indicate SE

7.4 Discussion

We have here shown that one of the main effects of HF-RSS is to increase the effectiveness of inhibition along the somatosensory pathway at both cortical and subcortical levels. However, the behavioural improvement (i.e., of STDT) only correlated with the changes in the measures tapping inhibition within S1 (i.e., R5-N20 and l-HFOs). This confirms the fact that STDT is crucially encoded in S1 and is dependent on intracortical inhibitory mechanisms (cf. chapter 5).

It has been previously suggested that both short latency paired pulse interactions at R5 and l-HFOs reflect activity in GABAa-ergic neurones that are known to produce feed-forward inhibition, at least at cortical level, of excitatory somatosensory inputs (Rocchi, Casula, et al., 2016). These neurones sharpen the temporal profile of the incoming input by preventing overlap with later-arriving dispersed inputs in the same pathway. Therefore, we speculate that repetitive activation of these neurons during HF-RSS increased the effectiveness of this feed-forward inhibition, thus increasing the suppression of N20 and P14 components of the SSEP produced by the second stimulus of a pair. However, HF-RSS may also increase the excitability of post-synaptic neurons responsible for N20 and P14 generation, consistent with the observed increase in amplitude of the cortical N20 (Hashimoto, Mashiko, & Imada, 1996) and in the P14 from the nucleus cuneatus (Cruccu et al., 2008). Nonetheless, increased amplitude of the SSEP did not correlate with changes in STDT suggesting that the change in temporal inhibition was the main factor influencing temporal discrimination, which is consistent with our previous results that $N20_{R5}$ and l-HFOs are the only two electrophysiological measures predicting STDT (cf. chapter 5).

Interestingly, HF-RSS also increased SICI in the APB but not in other muscles and left the unconditioned MEP unchanged, pointing to a focal transmission of HF-RSS effects to inhibitory mechanisms acting within the motor system. The lack of change of SICI in ADM is not entirely surprising according to the somatotopic organization of motor cortical input-output relationship described in previous investigations. Several authors have reported that in monkeys M1 receives sensory information from portions of limbs in close relation to the muscle to which it projects (Asanuma & Rosen, 1972; Rosen & Asanuma, 1972). Also in humans, there are extensive and somatotopic connections between S1 and M1 directly targeting layer V pyramidal tract neurons or relaying in MI cortical layers II/III (Kaneko, Caria, & Asanuma, 1994). Moreover, MEP amplitude is also modulated by stimulation of cutaneous fields close to the muscle involved (Classen et al., 2000; Tamburin, Manganotti, Zanette, & Fiaschi, 2001). It is also known that tetanic stimulation of S1 produces long-term potentiation in layers II/III of M1 (Keller, Iriki, & Asanuma, 1990; Sakamoto, Porter, & Asanuma, 1987). This could represent one pathway whereby HF-RSS might somatotopically increase excitability of the M1 GABAergic interneurons involved in SICI (Kujirai et al., 1993) and it is intriguing that SICI, N20 recovery curve as well as l-HFOs have been all suggested to reflect the activity of GABAergic interneurons (Kujirai et al., 1993; Murakami, Sakuma, Nomura, Nakashima, et al., 2008; Rocchi, Casula, et al., 2016).

Since HF-RSS was applied on skin closer to APB than ADM, it is plausible that modulation of SICI was clearer in APB. However, this does not explain why SICI in FDI was unaffected. The reason might be that TMS was centered over APB representation in M1; this means that activity in M1 evoked by TMS conditioning pulse was probably less effective in FDI

representation and thus the effects of HF-RSS might have gone undetected on the FDI.

In conclusion, we have shown that HF-RSS increases the effectiveness of inhibition at cortical and subcortical nodes of the somatosensory pathway in both sensory and motor domains. The augmented inhibition within S1 would explain the improvement in STDT while the increased amount of SICI might explain the previously reported motor performance induced by HF-RSS in the elderly (Kalisch et al., 2008, 2010). Arguably, this makes HF-RSS a suitable tool to potentially enhance inhibition in those disorders, including dystonia, where the latter is thought to be deficient (cf. chapter 1).

Chapter 8:
Abnormal inhibitory plasticity in cervical dystonia

The work presented in this chapter was originally submitted in the form of a research article: Erro R, Rocchi L, Antelmi E, et al. High frequency sensory stimulation in cervical dystonia: Evidence for defective inhibitory plasticity. 2017. Submitted.

8.1 Introduction

We have seen that HF-RSS is able to induce an improvement in STDT in healthy subjects (cf. chapter 6) and that such an improvement is correlated with an increased effectiveness of inhibition within S1 (cf. chapter 7). This makes HF-RSS an interesting tool to revert the neurophysiological and behavioural abnormalities observed in dystonia (cf. chapter 1 and 5).

The aim of the current experiment was hence to test whether this was the case in a group of patients with CD, the commonest form of adult-onset idiopathic dystonia. Since we also demonstrated that HF-RSS could increase the amount of SICI in healthy subject (cf. chapter 7) and this is known to be abnormal in dystonia, measures of motor excitability and inhibition were also gathered, as detailed below.

8.2 Methods

8.2.1 Participants

Twelve consecutive patients with a diagnosis of idiopathic isolated cervical dystonia according to current criteria (Albanese et al., 2013) were prospectively recruited from the outpatient clinics at the National Hospital for Neurology and Neurosurgery, Queen Square, London, UK. All patients were assessed at least 3 months after their last set of BoNT injections.

Twelve healthy volunteers with similar age (59.50±13.73 vs 62.17±9.80, HC vs CD; $p>0.05$) and gender distribution (3 vs 6 female, HC vs CD; $p>0.05$) and no family history for any neurological disorders served as HC.

8.2.2 Procedure

The procedure was the same as in the previous experiment (cf. chapter 7), but more electrophysiological tests were performed based on previous literature on dystonia. Thus, HF-RSS was applied on the right index finger. The STDT was collected before (T0) and after HF-RSS (T1) in the right index finger (i.e. target finger) and right thumb and left index finger (i.e., control fingers). At both T0 and T1, all subjects further performed an extensive electrophysiological battery tapping measures of sensory excitability and inhibition (SSEP, SSEP recovery cycle, SSEP lateral inhibition, HFOs) and motor excitability and inhibition (SICI and ICF), as detailed in Chapter 3. T0 and T1 electrophysiological measurements (i.e., SSEP vs TMS) were counterbalanced across subjects.

8.2.3 Statistical analysis

We first examined each variable for normality via the Shapiro–Wilk test, which was violated in most cases ($p<0.05$); therefore, non-parametric statistics were applied. Thus, Friedman test, Wilcoxon signed-rank test, and the Mann–Whitney U test were performed as appropriate. Data sets were first analysed in each group (HC and patients) separately; in fact, baseline differences between groups might have rendered interpretation difficult if both groups had been entered in the same analysis (Meunier et al., 2012). Then, possible correlations between behavioural and electrophysiological data were evaluated in both groups as a whole with the Spearman correlation analysis with Bonferroni correction. Moreover, since we were mostly interested in possible correlations between changes

induced by HF-RSS, and to further reduce the number of comparisons, each variable change was expressed as a ratio of measurements post/pre HF-RSS and entered in the Spearman's model. p<o.05 was deemed significant. Unless otherwise stated, data are given as mean ± standard deviation (SD). All analyses were implemented using STATA v.11 (STATACorp, USA).

8.3 Results

8.3.1 Somatosensory temporal discrimination threshold

As expected, at T0 CD patients had higher STDT than HC in all examined fingers (figure 1, p<.01). In HC, HF-RSS induced significant STDT changes (Friedman $\chi2=32.71$, p<.01). This was due to a significant STDT reduction (i.e. perceptual improvement) in the right index finger (z=3.10; p<.01), but not in the control fingers (p>0.05; fig. 8.1). In CD patients, HF-RSS also induced significant changes (Friedman $\chi2=32.71$, p<0.01), but with opposite direction with respect to HC. In fact, STDT increased in the right index finger (z=-2.35; p<0.01). Moreover, a significant STDT increase was also observed in the right thumb (fig. 8.1; z=-2.28; p<0.01) but not in the left index (z=0.598; p>0.05). Obviously, STDT values at T1 were higher in patients than in HC in all examined fingers (fig. 8.1; for all, p<0.01).

8.3.2 Somatosensory evoked potentials

Baseline stimulation intensity for SSEP recording, P14 and N20-P25 latency and amplitude were not different between patients and HC for stimulation of either right index finger or right thumb (all p>0.05; table 8.1). HF-RSS induced significant changes in SSEP N20-P25 amplitude (Friedman $\chi2 = 4.17$; p<0.05) and P14 amplitude (Friedman $\chi2 = 10.66$; p<.01) recorded from stimulation of the right index finger, but not of the right thumb (for both N20-P25 and P14 component Friedman $\chi2$ <0.33

and p>0.05). Post-hoc Wilcoxon signed ranks tests showed that amplitude of both N20-P25 (.61±.11 mcV vs .75±.11 mcV, T0 vs T1, z=-3.06; p<0.01) and P14 (.45±.09 mcV vs .52±.08 mcV, T0 vs T1, z=-3.06; p<0.01) recorded from right index stimulation significantly increased in HC after HF-RSS, whereas SSEP amplitude was unchanged in CD patients (for both N20-P25 and P14 z<0.58 and p>0.05). Thus, at T1 there were significant differences between groups in terms of N20-P25 amplitude (Mann–Whitney z=2.66; p<0.01) and P14 amplitude (z=3.52; p<0.01) from right index stimulation (table 8.1).

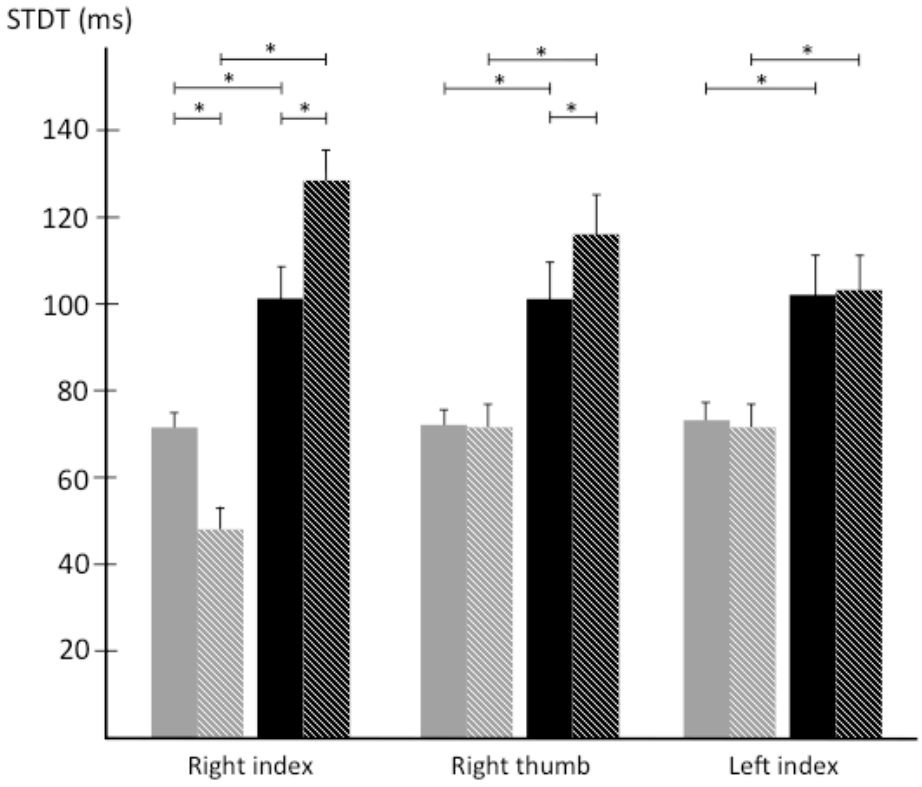

Figure 8.1 STDT in patients (grey columns) and healthy subjects (black columns), before (plain columns) and after (striped columns) HF-RSS. Starts indicate statistical significance (p<0.05). Vertical bars represent SE.

	HC	CD patients	p
INDEX FINGER			
Stimulation intensity, mA	7.35 (2.45)	7.27 (2.38)	>.05
N20-P25 latency T0, ms	22.86 (1.01)	22.67 (1.10)	>.05
P14 latency T0, ms	16.36 (0.70)	16.48 (0.62)	>.05
N20-P25 amplitude T0, µV	0.61 (.011)	0.57 (0.15)	>.05
P14 amplitude T0, µV	0.45 (0.09)	0.40 (0.07)	>.05
N20-P25 latency T1, ms	22.93 (0.97)	22.67 (1.04)	>.05
P14 latency T1, ms	16.40 (0.82)	16.42 (0.73)	>.05
N20-P25 amplitude T1, µV	0.75 (0.11)	0.57 (0.14)	**<.01**
P14 amplitude T1, µV	0.52 (0.08)	0.41 (0.06)	**<.01**
THUMB			
Stimulation intensity, mA	8.69 (3.12)	8.95 (2.97)	>.05
N20-P25 latency T0, ms	22.84 (1.02)	22.50 (1.09)	>.05
P14 latency T0, ms	16.25 (0.75)	16.34 (0.62)	>.05
N20-P25 amplitude T0, µV	0.64 (0.14)	0.57 (0.15)	>.05
P14 amplitude T0, µV	0.42 (0.05)	0.38 (0.07)	>.05
N20-P25 latency T1, µs	22.81 (1.00)	22.62 (1.04)	>.05
P14 latency T1, ms	16.26 (0.82)	16.26 (0.70)	>.05
N20-P25 amplitude T1, µV	0.64 (0.14)	0.57 (0.15)	>.05
P14 amplitude T1, µV	0.43 (0.07)	0.39 (0.08)	>.05
PAIRED STIMULATION			
N20 sum T0, µV	1.26 (.20)	1.15 (0.29)	>.05
N20 paired st. T0, µV	0.95 (0.18)	1.17 (0.29)	**<.01**
SIR_{N20} T0	0.73 (0.06)	1.01 (0.05)	**<.01**
P14 sum T0, µV	0.87 (0.11)	0.78 (0.14)	>.05
P14 paired st. T0, µV	0.64 (0.06)	0.79 (0.16)	**<.01**
SIR_{P14} T0	0.72 (0.08)	1.02 (0.08)	**<.01**
N20 sum T1, µV	1.38 (0.21)	1.14 (0.29)	**<.01**
N20 paired st. T1, µV	0.78 (0.14)	1.36 (0.31)	**<.01**
SIR_{N20} T1	0.55 (0.05)	1.02 (0.05)	**<.01**
P14 sum T1, µV	0.96 (0.12)	0.79 (0.12)	**<.01**
P14 paired st. T1, µV	0.49 (0.07)	0.97 (0.13)	**<.01**
SIR_{P14} T1	0.52 (0.07)	1.23 (0.08)	**<.01**

Table 8.1 SSEP results from single and paired (i.e., concomitant index finger and thumb stimulation) stimulation. Data are expressed as mean (SD). Significant p values are expressed in bold.

8.3.3 SSEP recovery cycle

At baseline, there were significant between-group differences as to R5-N20 (Mann–Whitney z=-1.88; p<0.05) and R20-N20 (Mann–Whitney z=-

2.48; p<0.05) but not as to R40-N20 (Mann–Whitney z=-1.86; p=0.063) or as to P14 recovery cycle at all ISIs (for all z>-1.82 and p>0.05). In HC, HF-RSS significantly enhanced inhibition (Friedman $\chi 2$ = 51.33; p<0.01). Specifically, this occurred for R5-N20 (Wilcoxon signed ranks z=2.98; p<0.01), R20-N20 (z=3.06; p<0.01), R40-N20 (z=3.06; p<0.01), R5-P14 (z=2.85; p<0.01), R20-P14 (z=3.06; p<0.01), and R40-P14 (z=3.06; p<0.01) (figure 8.2). In CD patients, HF-RSS also induced significant changes as to SSEP recovery cycle (Friedman $\chi 2$ = 50.97; p<0.01). At variance with HC, this was due to a reduction in inhibition as to R5-N20 (z=-2.83; p<.01), R20-N20 (z=-2.47; p<0.01), R5P14 (z==3.06; p<0.01), and R20P14 (z=-2.58; p<0.01) (figure 2). Consequently, there were significant differences between-groups at T1 in terms of both N20 and P14 recovery cycle at all ISIs (for all Mann–Whitney z<-4.163 and p<0.01; figure 8.2).

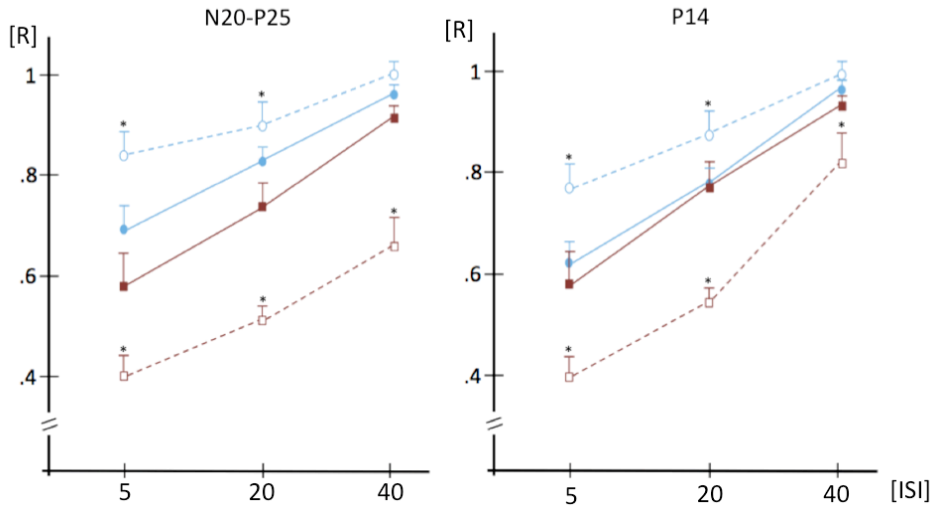

Figure 8.2 SSEP recovery cycles in patients (blue circles) and healthy subjects (red squares), before (plain squares/circles) and after (empty squares/circles) HF-RSS. Vertical bars represent SE. Only significant within-group comparisons are indicated with a star; for between-group comparisons see text. R: Ratio second/first SSEP, see text for details.

8.3.4 Sensory lateral inhibition

There were baseline differences between groups for both SIR_{N20} (Mann–Whitney z=-4.16; p<0.01) and SIR_{P14} (Mann–Whitney z=-4.16; p<0.01) with patients having a higher ratio than controls (table 8.1), which is indicative of less lateral inhibition. In HC, HF-RSS induced significant changes in both SIR_{N20} (Wilcoxon signed ranks z= 3.06; p<.01) and SIR_{P14} (Wilcoxon signed ranks z= 3.06; p<0.01). In both cases, HF-RSS reduced the ratio (SIR_{N20}: .73±.06 vs .55±.05 and SIR_{P14}: .72±.08 vs .52±.07, T0 vs T1) indicative on enhanced lateral inhibition. HF-RSS induced opposite results in CD patients for both SIR_{N20} (Wilcoxon signed ranks z= 3.06; p<0.01) and SIR_{P14} (Wilcoxon signed ranks z= 3.06; p<0.01). In both cases, the ratio was increased after HF-RSS (SIR_{N20}: 1.01±.05 vs 1.20±.05 and SIR_{P14}: 1.02±.08 vs 1.23±.08, T0 vs T1) suggestive of reduced lateral inhibition (table 8.1). Obviously, both SIR_{N20} (Mann–Whitney z=-4.16; p<0.01) and SIR_{P14} (Mann–Whitney z=-4.16; p<0.01) were significantly different between groups at T1 (table 8.1).

8.3.5 SSEP high frequency oscillations

No baseline differences were observed between groups as to both e-HFOs (Mann–Whitney z=1.44; p>0.05) and l-HFOs (z=0.46; p=0.06). HF-RSS induced significant changes of HFOs (Friedman $\chi 2$= 12.00; p<0.01) in HC, in whom both e-HFO (Wilcoxon signed ranks z=-3.06; p<0.01) and l-HFO area (z= z=-3.06; p<0.01) significantly increased (figure 8.3), suggestive of enhanced inhibition. HF-RSS induced significant changes also in CD patients (Friedman $\chi 2$= 5.3; p<0.05), but with an opposite pattern. In fact, both e-HFOs (Wilcoxon signed ranks z=2.27; p<0.05) and l-HFOs (z=2.82; p<0.01) significantly reduced after HF-RSS (figure 8.3). Consequently, there were significant differences at T1 between groups as to both e-HFOs

(Mann–Whitney z=4.02; p<0.01) and l-HFO (Mann–Whitney z=2.94; p<0.01) area.

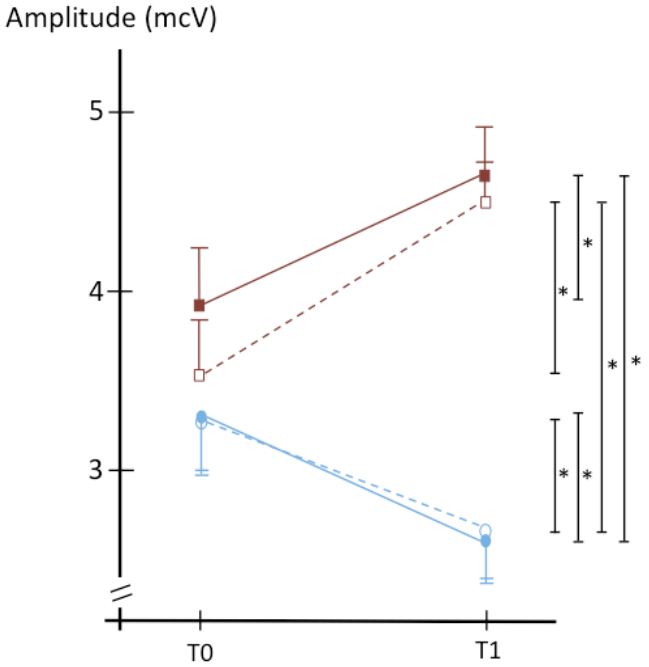

Figure 8.3 HFO in patients (blue circles) and healthy controls (red squares). Plain squares/circles indicate e-HFO and empty squares/circles indicate l-HFO. Vertical bars represent SE. Stars indicate statistical significance (p<0.05).

8.3.6 Corticospinal excitability

No differences were identified between groups as to RMT (Mann–Whitney z=-1.33; p>0.05), AMT (z=.20; p>0.05) and 1mV-int (z=.01; p>0.05)(table 8.2). MEP amplitude after single pulses were found significantly different between groups for the FDI (Mann–Whitney z=-2.51; p=0.01) and ADM (z=-3.24; p<0.01), but not the APB muscle (z=-0.26; p>0.05). In both former cases, patients had larger MEPs than HC (table 8.2).

HF-RSS did not induce any changes on MEP amplitude in any muscles in both HC (Friedman $\chi 2$= 4.03; p>.05) and CD patients (Friedman $\chi 2$= 4.09; p>.05). Thus, at T1 the same pattern was observed as at baseline, with patients having significantly larger MEP in the FDI (z=-2.74; p<0.01) and

ADM (z=-2.89; p<0.01), but not in the APB muscle (z=-.69; p>0.05) (table 8.2).

	HC	CD patients	p
RMT (%)	45.58 (9.66)	50.00 (8.2)	>.05
AMT (%)	39.88 (8.94)	41.08 (9.58)	>.05
1mV-int (%)	61.16 (12.34)	61.50 (11.45)	>.05
MEP_{APB} amplitude T0 (mV)	0.98 (0.21)	1.01 (0.19)	>.05
MEP_{FDI} amplitude T0 (mV)	0.95 (0.29)	1.27 (0.18)	**<.01**
MEP_{ADM} amplitude T0 (mV)	0.44 (0.13)	0.70 (0.15)	**<.01**
MEP_{APB} amplitude T1 (mV)	0.99 (0.23)	1.02 (0.14)	>.05
MEP_{FDI} amplitude T1 (mV)	0.91 (0.26)	1.24 (0.16)	**<.01**
MEP_{ADM} amplitude T1 (mV)	0.47 (0.15)	0.71 (0.18)	**<.01**

Table 8.2 Corticospinal excitability in HC and patients with CD. Data are expressed as mean (SD). Significant p values are expressed in bold.

8.3.7 Cortical inhibition in the motor system

At baseline, there were significant differences between groups for all muscles, with patients having higher ratios at all ISIs (for all, p<0.01; figure 8.4). In HC, HF-RSS induced significant changes in the APB (Friedman $\chi2$= 54.20; p<0.01), but not in the FDI or ADM muscles (for both $\chi2$<4.12; p>0.05). Specifically, the SICI reduced in the APB at ISIs of 70 (Wilcoxon signed ranks z=2.75; p<0.01), 80 (z=2.75; p<0.01) and 90 ms (z=2.35; p<0.01) (figure 8.4). In CD patients, HF-RSS induced significant changes of SICI in APB (Friedman $\chi2$= 49.43; p<0.01), FDI ($\chi2$= 12.94; p<0.05) and ADM muscle ($\chi2$= 30.12; p<0.01). As to APB muscle, SICI significantly increased at ISIs of 70 (Wilcoxon signed ranks z=-2.86; p<0.01), 80 (z=-2.71; p<0.01) and 90 ms (z=-2.90; p<0.01) (figure 8.4). As to FDI muscle, SICI significantly increased only at 90 ms (z=-2.28; p<0.01). As to ADM muscle, SICI significantly increased at ISIs of 80 (z=-2.51; p=0.01) and 90 ms (z=-2.75; p<0.01). Between-group comparisons at T1 showed that HC and CD patients differed in terms of SICI at all ISIs and in

all explored muscles (figure 8.4), with the exception of $SICI_{70}$ in the ADM muscle, where only a non-significant trend was observed (Mann–Whitney z=-1.88; p=0.06).

No differences were observed between groups in terms of LICI and ICF, either at T0 or at T1 (figure 8.5). HF-RSS did not induce any changes within groups (figure 8.5).

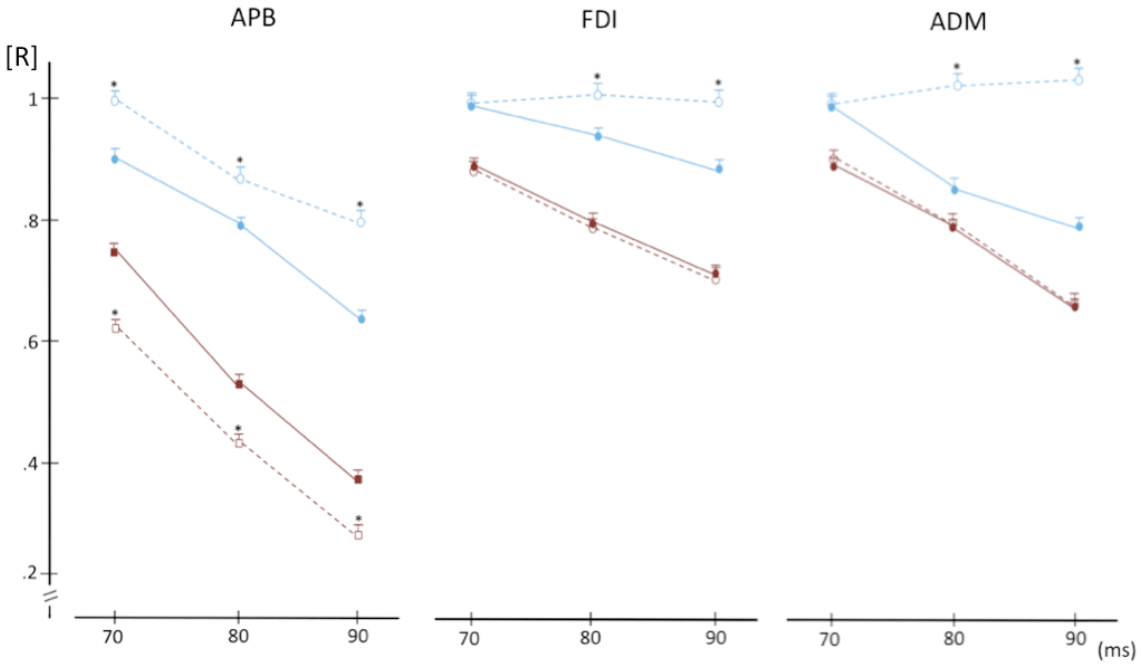

Figure 8.4 SICI in patients (blue circles) and HC (red squares), before (plain squares/circles) and after (empty squares/circles) HF-RSS. Vertical bars represent SE. Only within-group significant comparisons ($p<0.05$) are indicated with a star. For between-group comparisons see text. R: Ratio second/first MEP, see text for details.

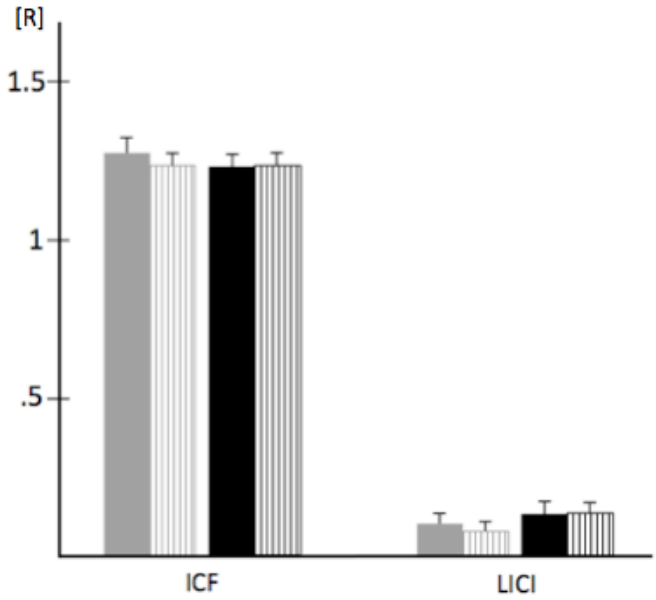

Figure 8.5 ICF and LICI in HC (grey columns) and patients (black columns) showing no differences either within or between groups at any time evaluation (plain: before- and striped: after HF-RSS). Vertical bars represent SE. R: Ratio second/first MEP, see text for details.

8.3.8 Correlations

In both groups, $STDT_{RATIO}$ on the right index finger was correlated with both $R5N20_{RATIO}$ (Spearman's rho: 0.653 and 0.713, HC and CD; both $p<0.01$) and $l\text{-}HFO_{RATIO}$ (Spearman's rho: -0.761 and -0.742; HC and CD; $p<0.01$). There was also a significant correlation between $l\text{-}HFO_{RATIO}$ and $R5N20_{RATIO}$ (Spearman's rho: -0.767 and - -0.692, HC and CD; $p<0.01$). No other significant correlations were observed between behavioural and electrophysiological measures.

8.4 Discussion

Baseline comparisons between groups largely replicated previous findings showing that patients with dystonia have higher STDT (Bradley et al.,

2010; Conte et al., 2014; Fiorio et al., 2007; Fiorio et al., 2008; Kimmich et al., 2014; Tinazzi et al., 2002; Walsh et al., 2007), reduced suppression of the recovery cycle of SSEP (Tamura et al., 2008), impaired lateral inhibition in both sensory (Antelmi et al., 2017; Tinazzi et al., 2000) and motor system (Beck et al., 2008; Sohn & Hallett, 2004) as well as reduced SICI (Y. Z. Huang et al., 2010; McDonnell et al., 2007), suggesting a widespread loss of inhibition in various areas of the CNS (Hallett, 2011).

Novel to the current study, however, is the difference in HF-RSS induced plasticity between groups. Thus, the main findings of the present study are that: 1) in HC, 45-min HF-RSS is able to potentiate several inhibitory mechanisms within the sensory system, the enhancement of some of which (i.e., suppression of the N20-P25 SSEP component at short ISI and l-HFO) accounting for the observed perceptual gain in terms of STDT, and the motor system as demonstrated by an increased amount of SICI; and 2) there is a paradoxical response to such a stimulation protocol in patients with CD. In fact, we observed a worsening of all aforementioned measures, indicating that the *responsiveness* of inhibitory circuitries to this type of stimulation is intrinsically abnormal in dystonia.

HF-RSS is a relatively novel technique that, at variance with other stimulation protocols able to induce associative plasticity, uses the physiological somatosensory pathway to target specific sensory areas with a very high topographic selectivity. In fact, the current and previous findings in healthy volunteers have shown that the behavioural consequences of HF-RSS are selectively confined to the stimulated area (Dinse et al., 2006; Erro et al., 2016; Godde et al., 1996; Godde et al., 2000; Pleger et al., 2003).

In their work in animals, Godde and colleagues (Godde et al., 1996) have demonstrated that HF-RSS increases the representational area of the stimulated digit in sensory cortex and increases the receptive field size of

individual cortical neurons. Furthermore, a functional MRI study in humans, showed that the representational cortical area of the stimulated finger enlarges after HF-RSS (Pleger et al., 2003). However, somewhat ambiguously, this protocol also enhances two-point discrimination (Dinse et al., 2006; Godde et al., 1996; Godde et al., 2000; Pleger et al., 2003). As the authors anticipated, an initial expectation might be that larger receptive fields would reduce perceptual acuity. However, we have previously demonstrated that HF-RSS can increase the efficacy of somatosensory inhibition (cf. Chapter 7) and it might well be that HF-RSS has two consequences, both of which are spatially limited to the area of stimulation: increased size of spatial receptive fields and increased effectiveness of somatosensory inhibition. The combination of these two effects can theoretically explain the perceptual gain better than either mechanism alone. In the spatial domain, increased spatial discrimination between stimuli would benefit both from larger receptive fields as well as increased effectiveness of inhibitory connections between adjacent fields. In the temporal domain, discrimination abilities might similarly benefit from engagement of larger numbers of neurons involved in temporal processing as well as augmented feed-forward somatosensory inhibition sharpening the temporal profile of excitatory somatosensory inputs (Rocchi, Casula, et al., 2016).

While our results show that HF-RSS may in fact increase the excitability of post-synaptic neurons responsible for N20 and P14 generation (Cruccu et al., 2008; Hashimoto et al., 1996), consistent with the observed increase in amplitude of the cortical N20 and of the P14 from the nucleus cuneatus, increased excitability of the SSEP did not correlate with changes in STDT suggesting that the increased efficacy of inhibitory mechanisms is required for a perceptual gain in temporal processing of somatosensory stimuli (cf. Chapter 7).

In keeping with this, we found that abnormal STDT values in dystonia correlated with specific measures of impaired intracortical inhibition, in line with previous findings (Antelmi et al., 2017), and that STDT worsened as long as such inhibitory mechanisms became less efficient, thus demonstrating that STDT is crucially dependent on these mechanisms.

SSEP suppression of the N20-P25 at short intervals (i.e., of 5 milliseconds) is thought to be primarily of cortical origin (Meyer-Hardting et al., 1983). There are many types of highly-specialized inhibitory interneurons in the cortex (Somogyi, Tamas, Lujan, & Buhl, 1998), and this temporal inhibition at short ISI may be carried out by a particular class of such interneurons. While HF-RSS induced functional changes at both subcortical (as demonstrated by the changes in the P14 recovery cycle as well as in the N20-P25 recovery cycle at longer ISIs), the correlations with STDT reached significance only with SSEP suppression at the shortest ISI, which suggests that the synaptic modulation of interneurons in upper cortical layers of S1 accounts for abnormal temporal processing. Analogously, l-HFOs represent activity of S1 inhibitory interneurons (Ozaki & Hashimoto, 2011), and such activity was paradoxically modulated by HF-RSS in patients with dystonia. Also in this case, the correlation with STDT changes was significant, hinting at an abnormal responsiveness of these interneurons, which are critically involved in temporal processing. Altogether, these layers of evidence demonstrate that the mechanisms regulating the activity of inhibitory interneurons are intrinsically abnormal, or in other words, that there is abnormal inhibitory plasticity within S1 in CD. Beyond the measures correlating with the behavioural outcome, this abnormal plasticity was observed with different types of sensory inhibition, including measures of lateral inhibition, and at different levels (i.e., cortical and subcortical levels) of the somatosensory pathway.

Few studies have previously suggested that cortical inhibitory plasticity might be abnormal in FHD (Meunier, Russmann, Shamim, Lamy, & Hallett, 2012; Tamura et al., 2009). Thus, Tamura et al. showed that the amplitude of the cortical P27 component of the SSEP was significantly higher in FHD patients than in HC after a paired associative stimulation (PAS) protocol, which consisted of peripheral electrical nerve stimulation and subsequent TMS over S1 (Tamura et al., 2009). Moreover, the authors found that baseline abnormal P27 suppression with paired pulses tended to normalize to the level of healthy subjects after PAS (Tamura et al., 2009). Although the authors suggested that the increased plasticity in S1 in FHD should be attributable to the disorganized inhibitory interneurons in upper cortical layers, they did not directly demonstrate that the plasticity of inhibitory interneurons was defective. In fact, they anticipated that the putative change in the inhibitory interneurons could not clearly explain the overall increased cortical excitability (Tamura et al., 2009). Supporting this view, we failed to shown increased SSEP amplitude in CD patients as *consequence* of decreased inhibition, suggesting that these changes need to be explained by different mechanisms, which warrants further investigations. Meunier et al. (Meunier et al., 2012) also hinted at the possibility that inhibitory cortical plasticity within S1 is deranged in FHD by showing that LAI, which reflects activity of somatosensory inputs to the motor cortex, was paradoxically increased after PAS. However, that PAS-induced changes of LAI might have been also driven by excessive plasticity induced in excitatory intracortical pathways within M1, which is often seen in dystonia (Quartarone et al., 2008; Quartarone & Pisani, 2011).

Thus, at variance with the aforementioned studies, we succeeded in showing a specific detrimental effect of HF-RSS on inhibitory mechanisms, clearly demonstrating a deranged inhibitory plasticity within the sensory system in dystonia.

A further fundamental question that remained unanswered thus far is whether the physiological abnormality observed within the sensory system represents a primary pathological condition or an adaptation process secondary to symptom manifestation. In fact, in both the aforementioned studies (Meunier et al., 2012; Tamura et al., 2009) the electrophysiological protocols were applied to a dystonic body region (i.e., affected hand in FHD patients). In this regard, CD might represent a better model since the effects of HF-RSS have been explored in an unaffected body region, which make us speculate that the observed deficits are primarily related to the pathophysiology of dystonia and are not merely consequential to abnormal posturing.

The measures of sensory lateral inhibition also worsened in patients after HF-RSS. While this did not directly correlate with the observed worsening of STDT, it might well be that these deficits in lateral inhibition accounted for the spread of detrimental STDT changes to an adjacent, non-stimulated, area (i.e., right thumb). This might be the behavioural sensory counterpart of the most known motor overflow observed in dystonia (Tinazzi et al., 2000).

Finally, we also demonstrated a focal transmission of these functional changes from the sensory to the motor system, as previously demonstrated in healthy volunteers (cf. Chapter 7). In fact, the responsiveness of inhibitory interneurons within M1 mirrored that of sensory interneurons. In healthy subjects, the effects of SICI were only detectable in APB and it is not entirely clear why SICI in FDI was not modulated. As speculated in the previous chapter, the reason might be that TMS was centered over APB representation in M1, hinting at the possibility that TMS-induced activity in M1 was less effective in FDI representation and thus the effects of HF-RSS were less clear. Whatever the reasons for this might be, the main result of the current experiment is

that HF-RSS induced opposite effects in CD patients with a reduction of the SICI, that was not only observed in the APB muscle but also in the FDI and ADM muscles. These results demonstrate that the inhibitory plasticity underpinning SICI (i.e. the responsiveness of the interneurons modulating the SICI) is also abnormal in dystonia and further confirm that there is abnormal spread of such plasticity, likely owing to loss of surround inhibition within M1 (Hallett, 2011).

As discussed in Chapter 7, the fact that the functional changes induced by HF-RSS were focally transferred from sensory to motor areas is in keeping with previous studies showing extensive and somatotopic connections between S1 and M1 directly targeting layer V pyramidal tract neurons or relaying in M1 cortical layers II/III (Kaneko et al., 1994). Moreover, a previous study using TBS protocols over M1 in healthy volunteers showed that changes in SICI were paralleled to those in HFOs (Murakami, Sakuma, Nomura, Nakashima, et al., 2008). Interestingly, a common neural mechanism has been suggested to be involved in the generation of SICI and HFOs (i.e. the activity of GABAergic inhibitory interneurons and their networks with pyramidal cells) (Murakami, Sakuma, Nomura, Nakashima, et al., 2008; Ozaki & Hashimoto, 2011). Since in our study SICI was prone to be influenced by HF-RSS as compared to MEPs from single pulses, we speculate that the changes in the effectiveness of synaptic connections among GABAergic inhibitory interneurons induced by HF-RSS might appear not only in the sensory cortex but also in the motor cortex via the cortico-cortical connections: As a result, SICI changed in parallel with HFOs. Moreover, since LICI, which is thought to be mediated by $GABA_B$ interneurons, was not influenced by HF-RSS, it might be that the latter targets selectively $GABA_A$ interneurons, upon which SICI is dependent (Di Lazzaro et al., 2007).

Taken together, these findings suggest that inhibitory plasticity within both sensory and motor system is primarily deranged in CD. The fact that HF-RSS targets primarily sensory areas, and the changes at this level are subsequently transferred to the motor system, would further suggest that spatially and temporally distorted sensory information could provide an altered assistance to the processing of motor programs (Tinazzi et al., 2000, 2003) and, perhaps, represent the *primum movens* for the development of dystonia.

Chapter 9:
Conclusions and future directions

This thesis describes the work I conducted during my PhD to investigate the physiological changes induced by a novel stimulation protocol, HF-RSS, in healthy subjects and patients with CD. My research has resulted in a better understanding regarding the effects of HF-RSS and further demonstrated that the effectiveness of inhibitory mechanisms (i.e., inhibitory plasticity) is abnormal in CD.

The main findings of this thesis are summarised below, together with the implications deriving from this work that, I hope, will foster future research not only into the field of dystonia, but also in other neurological disorders where inhibitory plasticity is supposed to be deficient.

9.1 HF-RSS is a novel NIBS protocol able to induce inhibitory plasticity

I have showed that one of the main consequences of HF-RSS is to potentiate the efficacy of inhibitory systems at various levels of the CNS and within both sensory and motor domains. At variance with other NIBS protocols that induce a net augmentation in inhibition through LTD-like changes on excitatory pathways (i.e., low frequency rTMS, cTBS, and cathodal tDCS), HF-RSS also augment inhibition, but through LTP-like changes on inhibitory interneurons, a phenomenon referred to as inhibitory plasticity.

Inhibitory plasticity is a relatively recent concept in the field of neuroscience (Kullmann, Moreau, Bakiri, & Nicholson, 2012). Until recently, research on neural plasticity focused almost exclusively on LTP/LTD-like changes at excitatory synapses on principal cells. It was assumed that inhibitory synapses on principal cells and the synapses

recruiting interneurons were not susceptible to plastic changes, as befits a role of inhibition in maintaining stable levels and accurate timing of neuronal activity. Instead, it is now clearly evident that inhibition is highly plastic, with multiple underlying cellular mechanisms, of which not all are entirely understood (for a review, see (Kullmann et al., 2012)). Owing to the diversity of neuronal circuitry in which inhibitory plasticity occurs, it is hard to propose a unifying theoretical model to explain its adaptive significance, being likely important for the regulation of excitability, generation of population oscillations, and precise timing of neuronal firing. In the mature neocortex, a closer look at the spatiotemporal profile of excitation and inhibition reveals that feed-forward inhibition and direct excitation of principal neurons in target structures are closely matched (Okun & Lampl, 2008; Priebe & Ferster, 2005; Wehr & Zador, 2003). Thus, there is strengthening of GABAergic synapses in response to postsynaptic activity and this calls for a mechanism for fine adjustment of inhibition to achieve ''detailed balance'' (Kullmann et al., 2012; Vogels & Abbott, 2009). While there is enough experimental evidence to support this argument as far as the visual modality is concerned (Kullmann et al., 2012; A. Maffei & G. Turrigiano, 2008; A. Maffei & G. G. Turrigiano, 2008; Yazaki-Sugiyama, Kang, Cateau, Fukai, & Hensch, 2009), there is only scarce information about the somatosensory modality. Nonetheless, it is postulated that, even in the somatosensory domain, if LTP at glutamatergic synapses on principal cells were not accompanied by an enhancement of inhibition, such interneuron-dependent functions as the temporal precision of information processing should be degraded (Kullmann et al., 2012).

The results of the work I performed in healthy subjects would nicely tie in with this hypothesis. Perceptual improvement induced by HF-RSS is achieved owing to the potentiation of inhibition, which counteracts over-excitation of target neurons, setting a new balance at higher efficiency.

There is evidence that some of the mechanisms underpinning inhibitory plasticity are NMDA receptor-dependent (Carvalho & Buonomano, 2009; Lamsa, Heeroma, & Kullmann, 2005; Pouille & Scanziani, 2001). Hence, the evidence that HF-RSS-induced plastic changes are also NMDA receptor-dependent does not necessarily imply that these changes only occur on excitatory synapses, as Godde et al. initially postulated (Godde et al., 1996). As extensively discussed above, it appears most likely that HF-RSS leads to both excitatory and inhibitory plasticity, the combination of which explains the perceptual gain better than either alone.

9.2 Inhibitory plasticity is defective in Cervical Dystonia

The experiment performed in patients with CD solidly shows that inhibitory plasticity is abnormal. The concept of maladaptive plasticity is well accepted in dystonia but it was, up to now, centered on plasticity occurring at excitatory synapses (Quartarone & Hallett, 2013; Quartarone et al., 2008; Quartarone & Pisani, 2011; Quartarone et al., 2005). Only two studies previously hinted at the possibility that inhibitory plasticity could be abnormal in dystonia (Meunier et al., 2012; Tamura et al., 2009), but none directly addressed the question of whether the *responsiveness* of inhibitory mechanisms was in fact altered.

Our novel findings open a new window for research since it remains to be established which mechanisms underpin at cellular level the paradoxical response observed in patients with dystonia. This might turn a suitable target for intervention.

Moreover, the deficient inhibitory plasticity was mainly demonstrated in the sensory domain and its behavioural consequences in a non-dystonic body region. This approach was deliberately chosen to avoid the confounding factor represented by the presence of overt dystonic manifestations, which was a major flaw in previous research (Meunier et

al., 2012; Tamura et al., 2009). However, our findings also imply that, while some of these defective inhibitory mechanisms are able to explain impaired sensory processing, they are *per se* not sufficient to produce dystonic symptoms. The speculation would be that on the background of abnormal sensory processing, which is arguably widespread to the entire body and genetically driven, additional factors trigger the development of dystonic manifestations in certain body parts and might, perhaps, further drive the spread from the initial site of symptoms to additional body regions. As previously suggested, these factors might be environmental and include insults to specific body regions that in turn would develop overt dystonia (Molloy et al., 2015; O'Riordan & Hutchinson, 2004; O'Riordan, Lynch, & Hutchinson, 2004). It is anticipated that this is not a linear, cause-effect relationship: For the development of dystonia a certain threshold, which is likely flexible to many genetic and epigenetic factors, must be reached. This framework would tie in with the evidence of reduced penetrance of genetic forms of dystonia and justify the common occurrence of sensory abnormalities in relative of dystonia patients or unaffected dystonia gene carriers (Fiorio et al., 2007; O'Dwyer et al., 2005; Walsh et al., 2007; Williams et al., 2017). It will be of interest to probe inhibitory plasticity using HF-RSS in these latter groups of subjects.

Finally, the fundamental idea behind HF-RSS is that modulation of the inherent statistics of sensory inputs can induce plasticity (Godde et al., 1996). If at the one end of the spectrum HF-RSS induce LTP-like changes, there is preliminary behavioural evidence that low-frequency RSS induces opposite results in healthy subjects, as demonstrated by impaired spatial sensory discrimination on the site of stimulation (Ragert, Kalisch, Bliem, Franzkowiak, & Dinse, 2008). This is not unexpected as high- and low-frequency rTMS similarly induce opposite results. At the current stage, it is unpredictable whether patients with dystonia will paradoxically benefit

from this type of stimulation. If in dystonia the intrinsic cellular mechanisms regulating both excitatory and inhibitory plasticity were abnormal, such a protocol would have detrimental effects, if any.

List of figures

Figure 5.1 Example of paired-pulsed SSEP in one representative healthy subject (left panel) and patient (right panel), showing less suppression (i.e., lateral inhibition) in the patient when the thumb and index finger were stimulated at the same time, while SSEP from individual fingers are similar……..42

Figure 5.2 Correlations between STDT and suppression of the N20 at 5 ms ISI (left panel) and l-HFO (right panel) in healthy subjects (red dots) and patients with CD (empty dots)……..43

Figure 6.1 Box whisker plot showing the distribution of STDT values on the stimulated finger (right index) and on the non-stimulated fingers (right thumb and left index) before (T0), 5 min (T1), 2.5 h (T2) and 24 h (T3) after the stimulation protocol in the young (top panel) and in the elderly group (bottom panel). Vertical bars represent SD. Stars indicate statistical significance ($p<0.05$). ……..51

Figure 7.1 STDT values on the stimulated finger (right index) and on the non-stimulated fingers (right thumb and left index) before (T0) and after (T1) the stimulation protocol. Vertical bars represent SD. Stars indicate statistical significance ($p<0.05$). ……..59

Figure 7.2 SSEP recovery cycle of N20-P25 (panels A-C) and P14 (panels D-F) components of SEP at ISIs of 5, 20 and 40 ms before (T0: panels A and D) and immediately after (T1; panels B and E) HF-RSS. HF-RSS increased the amplitude of unconditioned N20-P25 and P14 whereas it decreased the amplitude of paired pulse SSEP (i.e,, increasing the effectiveness of inhibition). For visualization purposes the raw signal was bandpassed between 20 and 500 Hz. Artefact from electric stimulus (at 0.05 s) was removed. Error bars indicate SE. ……..61

Figure 7.3 HFO before (left panel, T0) and immediately after (middle panel, T1) HF-RSS applied on the right index finger. HF-RSS induced a significant increase of both

early (p < 0.001) and late HFO area (p < 0.001). HFO area in the right panel is expressed in μV2 x 10-4. Artefact from electric stimulus (at 0.05 s) was removed. Asterisks indicate statistical significance. Error bars indicate SE. ……..62

Figure 7.4 Correlations between STDT, R5-N20 and l-HFO. The upper panels show a significant correlation between values of STDT and R5 (left) and between baseline values of STDT and l-HFO (right) at baseline. There was also a significant correlation between the changes induced by HF-RSS on STDT and the changes induced, respectively, on R5-N20 (lower left panel) and on l-HFO (lower right panel)……..63

Figure 7.5 Effects induced by HF-RSS on SICI on the APB. Raw signal from the APB of a single subject before (left panel) and after (middle panel) HF-RSS using different intensities of the conditioning TMS stimulus (CS) (70, 80 and 90% of AMT). HF-RSS induced an increase in SICI irrespective of the strength of the conditioning TMS pulse (all p values <001). Right panel shows SICI averaged among all subjects. Asterisks indicate statistical significance. Vertical bars indicate SE. ……..65

Figure 8.1 STDT in patients (grey columns) and healthy subjects (black columns), before (plain columns) and after (striped columns) HF-RSS. Starts indicate statistical significance (p<0.05). Vertical bars represent SE. ……..72

Figure 8.2 SSEP recovery cycles in patients (blue circles) and healthy subjects (red squares), before (plain squares/circles) and after (empty squares/circles) HF-RSS. Vertical bars represent SE. Only significant within-group comparisons are indicated with a star; for between-group comparisons see text. R: Ratio second/first SSEP, see text for details. ……..74

Figure 8.3 HFO in patients (blue circles) and healthy controls (red squares). Plain squares/circles indicate e-HFO and empty squares/circles indicate l-HFO. Vertical bars represent SE. Stars indicate statistical significance (p<0.05). ……..76

Figure 8.4 SICI in patients (blue circles) and HC (red squares), before (plain squares/circles) and after (empty squares/circles) HF-RSS. Vertical bars represent SE.

Only within-group significant comparisons ($p<0.05$) are indicated with a star. For between-group comparisons see text. R: Ratio second/first MEP, see text for details...78

Figure 8.5 ICF and LICI in HC (grey columns) and patients (black columns) showing no differences either within or between groups at any time evaluation (plain: before- and striped: after HF-RSS). Vertical bars represent SE. R: Ratio second/first MEP, see text for details. ……79

List of tables

Table 1.1 Summary of the behavioural/psycophysical evidence for abnormal sensory processing and/or sensorimotor integration
(Modified from Avanzino et al., 2015)
Abbreviations not present in the abb. list: TVR=Tonic Vibration Reflex; RHI=Rubber Hand Illusion.....19

Table 5.1 Summary of the demographic, clinical, behavioural and electrophysiological features in HC and patients. Data are expressed as mean±SD, unless otherwise specified. Significant values are indicated in bold. 40

Table 8.1 SSEP results from single and paired (i.e., concomitant index finger and thumb stimulation) stimulation. Data are expressed as mean (SD). Significant p values are expressed in bold. 73

Table 8.2 Corticospinal excitability in HC and patients with CD. Data are expressed as mean (SD). Significant p values are expressed in bold. 77

Abbreviations

ADM: Abductor digiti minimi muscle

AMT: Active motor threshold

APB: Abductor pollicis brevis muscle

BoNT: Botulinum neurotoxin

BPS: Blepharospasm

CD: Cervical dystonia

CNS: Central nervous system

CSP: Cortical silent period

cTBS: Continuous TBS

DBS: Deep brain stimulation

EEG: Electroencephalography

e-HFO: Early HFO

EMG: Electromyography

FDI: First dorsal interosseous muscle

FHD: Focal hand dystonia

HC: Healthy controls

HFO: High frequency oscillations

HF-RSS: High frequency RSS

ICF: Intra cortical facilitation

ISI: Inter stimuli interval

iTBS: Intermittent TBS

LAI: Long afferent inhibition

l-HFO: Late HFO

LICI: Long intra-cortical inhibition

LTD: Long term depression

LTP: Long term potentiation

MEP: Motor evoked potentials

MRI: Magnetic resonance imaging

NIBS: Non-invasive brain stimulation

PAS: Paired associative stimulation

RF: Receptive field

RSS: Repetitive sensory stimulation

rTMS: repetitive TMS

SAI: Short afferent inhibition

SD: Standard deviation

SE: Standard error

SICI: Short intra-cortical inhibition

SIR: Spatial inhibition ratio

SSEP: Somatosensory evoked potentials

STDT: Somatosensory temporal discrimination threshold

TBS: Theta burst stimulation

TWSTRS: Toronto western spasmodic torticollis rating scale

tCS: Transcranial current stimulation

TMS: Transcranic magnetic stimulation

TPDT: Two-point discrimination threshold

Peer-reviewed publications

List of the author's publications (chronological order) in scientific journals in relation to dystonia during the doctoral program (those not included in this thesis are also listed).

1. Erro R, Rubio-Agusti I, Saifee TA, et al. Rest and other types of tremor in adult-onset primary dystonia. J Neurol Neurosurg Psychiatry. 2014;85:965-8
2. Baschieri F, Batla A, Erro R, et al. Paroxysmal exercise-induced dystonia due to GLUT1 mutation can be responsive to levodopa: a case report. J Neurol. 2014;261:615-6.
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4. Erro R, Sheerin UM, Bhatia KP. Paroxysmal dyskinesias revisited: a review of 500 genetically proven cases and a new classification. Mov Disord. 2014;29:1108-16
5. Erro R, Bhatia KP, Hardy J. GNAL mutations and dystonia. JAMA Neurol. 2014;71:1052-3.
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9. Mencacci NE, Erro R, Wiethoff S, et al. ADCY5 mutations are another cause of benign hereditary chorea. Neurology. 2015;85:80-8

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14. Domingo A, Erro R, Lohmann K. Novel Dystonia Genes: Clues on Disease Mechanisms and the Complexities of High-Throughput Sequencing. Mov Disord. 2016;31:471-7
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19. Antelmi E, Erro R, Rocchi L, et al. Neurophysiological correlates of abnormal somatosensory temporal discrimination in dystonia. Mov Disord. 2017;32:141-148.
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