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IDIOPATHIC ISOLATED FOCAL DYSTONIA: *FROM IMPAIRED INHIBITION TO MODULATION OF DYSTONIC ACTIVITY DURING SLEEP*

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Esame finale anno 2017

"In tutto c'è qualcosa che resta insondato, perché siamo caduti nell'abitudine di ricordare, ogni volta che usiamo i nostri occhi, quello che gli altri prima di noi hanno pensato su ciò che stiamo osservando. Anche l'oggetto più insignificante ha un che di sconosciuto. Tocca a noi scoprirlo. Per descrivere un fuoco ardente o un albero in una pianura, dobbiamo rimanere dinanzi a quel fuoco o a quell'albero finché per noi essi non assomiglino a nessun altro fuoco o albero"

Guy de Maupassant

L'approccio "*antipodale"* della descrizione scientifica (biologica e neurofisiologica) e di quella fenomenologica emerge chiaramente da questo scritto "ante-litteram" (rispetto alla corrente fenomenologica) di Guy de Maupassant. Eppure quanto Maupassant dice può essere in parte applicato alla scienza, giacché per scorgere lo sconosciuto, bisogna in *senso lato* applicare il concetto di *epochè* husserliana. La differenza rispetto a una mera descrizione fenomenologica, sta nel fatto che l'*epochè* (intesa in termini non strettamente husserliani, ma come "conoscenza attuale-bagaglio culturale" relativo a un fenomeno) deve rappresentare comunque un punto di partenza e che, *nell'osservare con un occhio nuovo* un fenomeno, bisogna comunque conoscere alla perfezione tutto ciò che è stato già detto o fatto al fine di indagare lo stesso.

E.A.

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Chapter 1

Idiopathic isolated cervical dystonia

GENERAL INTRODUCTION

Dystonia over the years

Dystonia is a chronic movement disorder characterised by an aberration in the control of movement, with subsequent co-contraction of agonist and antagonist muscles causing repetitive and twisting movements, and abnormal postures.

We can find a first description calling to mind dystonia in the "Divina Commedia", when Dante Alighieri described the punishement of the fortune tellers and diviners who, for having looked too far forward, were then obliged with their heads twisted backwards (Divina Commedia, 1315; Inferno; Circle eight, *Bolgia* four).

And when I looked down from their faces, I saw
that each of them was hideously distorted
between the top of the chest and the line of jaw;
for the face was reversed on the neck, and they came on
backwards, starting backwards at their loins,
for to look before them was forbidden. Someone
sometime, in the grip of palsy may have been
distorted so, but never to my knowledge;

Dante Alighieri

The first medical description of cervical dystonia was probably by Tulpius, in his *Observationes Medicae* (1672), while the first description of a generalized dystonia was likely given by Gowers (1888) under the name of "tetanoid chorea", which he used to describe some of the clinical

features of patients affected with Wilson's disease. Then in 1908, Schwalbe reported the first description of a family with three affected children presenting features likely resembling those of a primary generalized dystonia.

The term however was firstly coined by Oppenheim in 1911 and in the same year Flatau and Sterling (1911) pointed out a likely inherited nature of the disorder.

At the beginning, patients with dystonia were often considered to be hysterical but later the identification of the gene locus for Oppenheim dystonia (Ozelius et al., 1989) and the observation of an autosomal dominant pattern of transmission in the Ashkenazi Jewish population (Bressman et al., 1989) pointed to its organic nature.

The classification of dystonia has evolved over the years and in 2013 an international panel of experts provided the last consensus update on the definition, phenomenology and classification of dystonia (Albanese et al., 2013). According to the latest consensus update "*dystonia is a movement disorder characterized by sustained or intermittent muscle contractions causing abnormal… movements, postures …. Dystonia is often initiated or worsened by voluntary action and associated with overflow muscle activation*". Previously, dystonic syndromes were classified along three main axes: etiology, age at onset, and body distribution (Fahn, 2011; Albanaese et al., 2011). The new classification encompasses only two axes, i.e. 'clinical characteristics' and 'etiology'.

Axis I regards clinical characteristics, namely age at onset, body distribution, temporal pattern, coexistence of other movement disorders and other neurological manifestations. The age at onset was subdivided into infancy (birth to 2 years), childhood (3–12 years), adolescence (13–20 years), early adulthood (21–40 years) and late adulthood (>40 years). The body distribution is described as focal, segmental, multifocal, generalized or hemidystonia. The temporal pattern includes both the disease course, which may be static or progressive, and the variability of

symptoms, which may persist, fluctuate diurnally or occur only on specific actions or in paroxysms. Associated features distinguish dystonia combined with another movement disorder (e.g., myoclonus-dystonia) or with other neurological or systemic manifestations.

Axis II regards etiology and namely those related to the nervous system pathology (evidence of degeneration/structural lesions/neither) and those due to inherited or acquired causes, respectively acquired forms (such as perinatal brain injury, infections, drugs among others) or idiopathic (sporadic/familial), which may be reclassified as inherited if new genes are recognized. The term 'primary' was replaced by the term 'isolated dystonia' to describe cases in which dystonia is the only motor feature, apart from tremor. The previously called heredodegenerative dystonia and dystonia plus syndromes (e.g., myoclonus-dystonia) are now named 'combined dystonia', therefore referring to phenomenology rather than etiology.

Focal dystonia

In June of 1975, at the International Symposium on Dystonia in New York City chaired by Stanley Fahn and Roswell Eldridge, David Marsden firstly reported on focal and sporadic forms (for instance, cervical dystonia, blepharospasm, oromandibular dystonia and writer's cramp), proposing these disorders as '*formes frustres*' of generalized dystonia.

Adult-onset idiopathic isolated focal dystonia (AOIFD) consists in dystonia involving the neck (cervical dystonia), the upper face (blepharospasm), the mouth and jaw (oromandibular dystonia), the larynx (laryngeal dystonia), or a limb (e.g., writer's cramp and other focal hand dystonia).

Cervical Dystonia

Cervical dystonia, characterized by sustained or intermittent neck muscle contractions causing abnormal head movements, is the most common form of AOIFD, with a prevalence ranging from 4.98 per 100,000 (Steeves et al., 2012) to 120 per million and a female preponderance (Donaldson et al., 2012). CD usually develops around one's forties and the risk of spreading is around 15% over 5 years (Jinnah et al., 2013). It may be sporadic or familial. A clinical classification is based on the type of movement and position of the head in the affected patients, the most common type being rotational torticollis (>50%). Involuntary neck muscles activity indeed may result in different patterns, i.e. torticollis when the head turns to one side (right or left), laterocollis when head tilts to one side, retrocollis when it tilts upward, or anterocollis if downward. Patients often show a combination of various abnormal patterns, even when a predominant component can be identified. A jerky tremor of the head is a common overlapping feature (Erro et al., 2014; Defazio et al., 2015) and overflow to the shoulder and to the contiguous arm is also observed.

Various sensory tricks, such as touching the contralateral face but also ipsilateral in the direction of head rotation, can improve the involuntary neck movements albeit temporarily. As in other forms of focal dystonia, stress exacerbates whereas relaxation improves the symptoms of CD.

Idiopathic CD is the most common form, although rare cases of CD due to basal ganglia lesions have been reported (Albanese et al., 2013).

Idiopathic CD is the result of the combination of genetic make-up and environmental risk factors.

Different genes have been linked with CD, but commonly, even if a familial link may be seen, the genetic analyses are negative, indicating that further unknown genes and the interaction with other risk factors and modifiers may be associated with this disease. Less frequently, CD may represent the onset feature or an associated feature of other conditions, like a parkinsonian syndrome (as for anterocollis or retrocollis in multiple system atrophy) or more complex

heredodegenerative diseases. It can be due to drug exposure, mainly dopamine receptor antagonists (Cardoso, 2008), to a trauma (van Rooijen et al., 2011), to an autoimmune disease (Baizabal-Carvallo and Jankovic 2012) or to a focal lesion (LeDoux, 2003), while when fixed it might be due to syrinx or psychogenic causes (Schrag et al., 2004; Hawley and Weiner, 2011).

Isolated CD, as said, is mainly an idiopathic condition. Few genes may present at first with CD, but usually in these contexts the clinical picture is broader and encompasses additional clinical signs. In patients with onset around midlife, the first gene to exclude is *THAP1* (thanatos associated protein domain containing, apoptosis associated protein 1) related to DYT6 (Almasy et al., 1997; Fuchs et al., 2009). Inheritance is autosomal dominant with a reduced penetrance, which is around 60% (Saunders-Pullman et al., 2007). The clinical spectrum of *THAP1* mutations varies. Presentations with oromandibular, cranio-cervical or laryngeal dystonia are common, but presentations with focal dystonia of the limbs, segmental or generalized dystonia are all described in the literature.

Recently, however, an additional four more genes have been described as cause of adult onset primary pure dystonia. The first gene to appear on the scene was *CIZ1* (Cip1- interacting zinc finger protein 1; DYT23) (Xiao et al., 2012). It causes mainly focal CD developing in mid-to-late life, without subsequent generalization. However, this has not been replicated in other cohorts.

Mutations of the anoctamin 3 gene (*ANO3*) were recently identified to cause autosomal dominant isolated dystonia and have been assigned to the locus dystonia-24 (DYT24) (Charlesworth et al., 2013). Age at onset ranged from early childhood to one's forties. The predominant phenotype was tremulous CD, whereas cranial and laryngeal dystonia were present to a variable degree. Mild dystonia of the arms might be observed in the arms, while generalization has not been reported. Tremor involving the arms and the head instead has been described as a consistent feature.

TUBB4A has recently been identified by two groups independently as the cause of DYT4 dystonia, also known as whispering dysphonia (Lohmann et al., 2012; Hersheson et al., 2013). However, this mutation seems rare and the dystonia usually generalized over the years. A cue is the association with the "hobby horse" gait.

Mutations in *GNAL*, encoding guanine nucleotide-binding protein, alpha activating activity polypeptide, olfactory type Ga(olf), which is involved in dopamine (D1) signaling, have recently been identified (Fuchs et al., 2013) as a cause of autosomal dominant primary dystonia. It mainly results in craniocervical dystonia, with rare (10%) generalization. Dystonic head tremor might be present. Some patients had laryngeal onset or developed spasmodic dysphonia. A distinguishing feature may be the association with hyposmia (Vemula et al., 2013).

Finally, even if the typical presentation of DYT1 is childhood-onset dystonic posturing of the foot or leg, with subsequent generalization, late-onset or milder forms of the disorder have been reported (Jamora et al., 2006).

Treatment

Initially, CD was treated with oral medications or surgical interventions, with frequent disappointing outcomes. Nowadays, chemodenervation using botulinum toxin (BoNT)-A has become the cornerstone of treatment for CD, with a good safety and efficacy profile. Surgical treatment, particularly pallidal neurostimulation, may be considered for patients with severe CD refractory to the combination of oral drugs and chemodenervation, while adjuvant physiotherapy might be proposed regardless of the therapeutic option.

Oral treatment (including anticholinergic agents, γ-aminobutyric acid -GABA mimetic agents, dopamine receptor antagonists, dopamine-depleting agents and even dopamine receptor agonists) is limited in efficacy and is mainly based on empirical evidence. Particularly,

anticholinergic medications, mainly trihexyphenidyl, might be useful and are generally well tolerated but they should be increased slowly, in order to avoid side effects. Usually, it is worth starting with a 2 mg tablet of trihexyphenidyl, one-half tablet twice a day and slowly increasing the dosage over several weeks up to 80 mg/ day divided into 3 doses. Larger doses may give dose-related drowsiness, confusion, or memory difficulty limiting the medication's usefulness.

Once the efficacy of BoNT had been demonstrated in the 1980s, it rapidly assumed a place as the treatment of choice for this condition (Albanese et al., 2010; Albanese et al., 2015).

A systematic review supports this and shows that BoNT is the most effective treatment for reducing dystonic symptoms in patients with focal dystonia (Zoons et al., 2012).

The benefits from BoNT can be sustained for decades and no permanent adverse effects of BoNT have been reported (Jankovic, 2006, 2013). The occurrence of blocking antibodies decrease is rare (Ramirez-Castaneda et al., 2013) and in this case an immunologically distinct type of BoNT may be used.

Deep brain stimulation (DBS) for dystonia has been performed since 1977 for CD and since 1999 for generalized dystonia (Kurnar et al., 1999). Internal globus pallidus (GPi) is the target in class I and II studies, which are level 1 evidence (Vidailhet et al., 2013). It is considered effective both for generalized and segmental dystonia. Younger age at surgery (< 21 years) and shorter duration of symptoms (< 15 years) are predictive of a better outcome. As regards DYT6, Vidailhet et al. (2013) showed that the site of dystonia itself is a better predictor than the genetic status, as patients with spasmodic dysphonia and cranial involvement tend to have a limited response to DBS.

Neurophysiology

Dystonia is the result of a disruption within a motor network, likely related to an interaction between the genetic make-up and the environmental modifiers. However, even if a large bulk of knowledge about dystonia has been produced in the recent years, the neurophysiopathological mechanisms underlying dystonia are still arcane and particularly it is still unclear what is correlative and what endophenotipic (Hallet, 2011).

Three core underlying mechanisms seem to drive abnormalities in dystonia (Quartarone and Hallet, 2013): loss of inhibition (Hallet and Rothwell, 2011), sensory dysfunction (Quartarone et al., 2006a; Tinazzi et al., 2009) and disruption of homeostatic plasticity, with a prevailing facilitation of synaptic potentiation and loss of synaptic inhibitory processes (Quartarone and Pisani, 2011).

Loss of inhibition can be easily read from clinical observations by observing the phenomena of overflow dystonia, of mirror dystonia and of geste antagoniste. Several neurophysiological findings further support this notion. By using transcranial megnetic stimulation (TMS), abnormal inhibition has been reported at several levels of the nervous system, i.e. at cortical level (Ridding et al., 1995; Huang et al., 2004), but also at the brainstem and at the spinal levels (Tish et al., 2006a and b) and despite unilateral symptoms, abnormal findings have been reported in both hemispheres (Ridding et al., 1995; Huang et al., 2004). The most consistent abnormalities among different studies are reduced short interval intracortical inhibition (SICI) and reduced motor surround inhibition (mSI) (Hallet, 2011).

Loss of inhibition seems to involve also the sensory system. In dystonic patients, impaired performances have been reported both in spatial discrimination (Bara-Jimenez et al., 2000a) and temporal discrimination (Bara-Jimenez et al., 2000b; Tinazzi et al., 2002; Molloy et al., 2003; Fiorio et al., 2003 e 2008; Scontrini et al., 2009). Abnormalities involving the sensory pathway were further proved by neurophysiological measurements, including recording of the

somatosensory evoked potentials (Bara-Jimenez et al., 1998), magnetoencephalography (Meunier et al., 2001) and functional brain magnetic resonance (Butterworth et al., 2003) or by studying somatosensory evoked potentials with dual or double stimulation (Tinazzi et al., 2000; Frasson et al., 2001; Tamura et al., 2008).

Sensorimotor integration also seems to be affected, and particularly the contingent negative variation, which is the EEG activity between two sensory stimuli (first warning and second commanding) that triggers a movement, it has been found to be abnormal prior to hand movements but not to neck movements in focal hand dystonia (Ikeda et al,. 1996), and prior to neck movements but not to hand movements in neck dystonia (Kaji et al., 1995).

Surround and lateral inhibitions are supposed to cause a breakdown in the circuits involved in the encoding of motor memories, therefore promoting the generation of abnormal motor engrams. In that regard, it appears deducible how intensive training might represent a causal trigger. Indeed, the mechanisms of long-term potentiation-like and long-term depression-like facilitatory and inhibitory effects on TMS motor evoked potentials have been reported to further enhance abnormal neurophysiological findings and symptoms in dystonic patients (Quartarone et al., 2003; Weise et al., 2006). Moreover, it has emerged clearly how abnormalities pertain not only to the neural circuits affected with dystonia but to the entire sensorimotor system (Quartarone et al., 2005; Quartarone et al., 2006; Quartarone et al., 2008).

List of the PhD projects.

During the three years of my PhD I have focused my research on dystonia with the aim to further analyze this fascinating disorder from a neurophysiological and clinical point of view.

Three main aims/projects have guided my studies:

- To analyze the pathophysiology of tremor in dystonia (Chapter 2);
- To analyze the pathophysiology of sensory abnormalities in dystonia (Chapter 3);
- To analyze the role of sleep in dystonia and the pattern of dystonic movements during the different state of beings (i.e., wakefulness and different sleep stages) (Chapter 4);

All the three study designs have been performed in patients with CD, being the most common type of isolated idiopathic dystonia.

The first two studies have been performed at the Sobell Department of Motor Neuroscience and Movement Disorders, Institute of Neurology, University College London, London, UK in Professor John Rothwell's laboratories. All patients were examined in the outpatient' clinic of Professor Kailash Bhatia.

The last study has been conducted at IRCSS, Institute of Neurological Sciences, Bologna, Italy of the Department of Biomedical and Neuromotor Sciences, Alma Mater Studiorum, University of Bologna, Bologna, Italy. All the patients were examined in the outpatient clinic of Professor Rocco Liguori.

Chapter 2

FIRST STUDY

The role of the cerebellum in dystonic tremor*

*Published as: **Antelmi E**, Di Stasio F, Rocchi L, et al. Impaired eye blink classical conditioning distinguishes dystonic patients with and without tremor. **Parkinsonism Relat Disord 2016**;31:23-7.

Background

Tremor is a common feature of dystonia (Erro et al., 2014; De Fazio et al., 2015), with a prevalence ranging from 11% to 87%, depending on the different cohorts (Erro et al., 2014; De Fazio et al., 2015). So far, dystonic tremor has frequently been misdiagnosed as essential tremor or Parkinson disease. The frequent misdiagnoses are likely due to the lack of markers for the differential diagnosis of tremulous conditions, and highlight the difficulty with the clinical diagnosis of dystonia. Isolated head tremor, presentation of head tremor before arm tremor and more severe head tremor than arm tremors are virtually all manifestations of dystonic tremors (Erro et al., 2014; De Fazio et al., 2015). An interesting clue is that in essential tremor, head tremor often disappears when the patient lies down but persists in CD, supporting the notion that tremor in ET is a postural tremor that dissipates when a patient lies down (Agnew et al., 2012). Patients presenting with isolated voice tremor predating the onset of hand tremor, and being more severe than hand tremor, are considered to be affected with "tremulous dystonia" (Erro et al., 2014). Usually dystonic tremors have irregular amplitudes and superimposed jerks. Rest tremor may occur in dystonia, but it is mostly unilateral or asymmetric and, remarkably, it does not have re-emergent tremor (Erro et al., 2014). Additional peculiarities revealing the underlying dystonic nature of tremor are: the position/task specificity, the jerkiness, the presence of tremor-flurries, thumb hyperextension, the pronation-supination type rather than vertical, the absence of a remarkable response to levodopa and the static rather than progressive disease (Erro et al., 2014). Tremor seems more frequent in adult onset patients with cervical involvement and segmental or multifocal dystonia (De Fazio et al., 2015).

In patients with CD, according to the classification of tremor in dystonia, (Deuschl, 2003) tremor may manifest in a body part affected by dystonia (dystonic tremor) or in body parts unaffected by dystonia (tremor associated with dystonia).

The pathophysiology of tremor in dystonia however is still largely elusive (De Fazio et al., 2015). Several studies have been conducted trying to disentangle this issue by means of neurophysiological findings. Münchau et al. (2001) by investigating spinal circuitry reported on abnormally reduced presynaptic reciprocal H reflex inhibition in patients with severe and early onset harm tremor, when compared to the milderly affected and to patients with ET. The blink reflex R2 recovery cycle showed higher brainstem excitability in patients with DT when compared to patients affected with ET (Nisticò et al., 2012 a-b). Finally, while the somatosensory temporal discrimination threshold has been reported to be abnormal both in ET and DT, temporal discrimination movement threshold was reported as a discrete feature of DT (Tinazzi et al., 2013). Besides, neurophysiologic studies in patients implanted with DBS in the GPi proposed that excessive synchronization in the frequency range of 3–18 Hz may be responsible for generating dystonic symptoms including tremor (Liu et al., 2008). It remains unclear, however, why GPi DBS improves dystonia but fails to improve tremor in dystonic patients. Yet, even confirming abnormal brainstem excitability and inhibitory processes, which are well known features of dystonia itself, there are no studies comparing neurophysiological findings in dystonic patients with and without tremor.

Dystonia is currently considered as the result of a network impairment (Quartarone and Hallet, 2013), within which the cerebellum has been recently suggested to play a pivotal role (Prudente et al., 2014). Structural changes were observed in CD in the anterior and posterior cerebellum (Piccinin et al., 2014) and in motor areas connected to the cerebellum, like the premotor and supplementary motor areas, globus pallidus, striatum, and thalamus (Draganski

et al., 2003; Colosimo et al., 2005; Egger et al., 2007; Obermann et al., 2007; Prell et al., 2013). Cerebellar dysfunction could also be claimed to explain certain behavioral deficits observed in patients with dystonia, such as impairments in movement timing (Bares et al., 2007; Bares et al., 2011; Filip et al., 2013; Avanzino et al., 2013).

Recently, by using functional MRI activation analysis, connectivity analysis, and voxel-based morphometry in patients with CD, a miscommunication between the basal ganglia and the cerebellar loops has been found, with decreased activation in the posterior cerebellar lobules as well as in the premotor areas, in the associative parietal cortex, and in visual regions (Filip et al., 2017). Patients showed also decreased cerebellar connectivity with bilateral basal ganglia structures and the dorsolateral prefrontal cortex (Filip et al., 2017).

To further stress a cerebellum involvement in dystonia, and particularly in dystonic tremor, microscopic cerebellar changes have been reported in patients with dystonia and tremor (Ma et al., 2012) and stimulation applied to Vim (the main target of cerebellar projections to the thalamus) have been reported to improve tremor in dystonia (Hedera et al., 2013).

Overall, the cerebellum and inferior olives are known to play a critical role in the pathophysiology of action-induced tremors (Raethjen and Deuschl, 2012) and therefore it is possible that a cerebellar dysfunction may contribute somewhat also to dystonic tremor.

A great body of literature reports on eye blink classical conditioning (EBCC) as a type of associative learning tightly linked to the cerebellum (Gerwig et al., 2007). Indeed, animal models documented the role of the cerebellar output, via interpositus neurons, in modulating eyeblink learned responses (Thompson, 2005), along with other structures, such as the hippocampus (Delgado-García and Gruart, 2006) and the amygdala (Boele et al., 2009). In humans, both cerebellar lesions and functional brain imaging data provide evidence that the cerebellum plays a pivotal role in classical conditioning, along with the brainstem circuitry and

likely with the diencephalic structures (Woodruff-Pak et al., 2001; Dimitrova et al., 2009; Berry and Hoffmann, 2011).

EBCC has been widely used to study the cerebellum in different diseases and it has been reported to be impaired in patients with ET (Kronenbuerger et al., 2007) and neuropathic tremor (Schwingenschuh et al., 2013).

As long as dystonic diseases are concerned, this paradigm has been reported to be normal in patients with generalized dystonia due to *TOR1A* (DYT1) and *THAP1* (DYT6) mutations (Sadnicka et al., 2015), as well as in patients with secondary dystonia (Kojovic et al., 2013) and a paradigm testing a fundamental cerebellar computation, i.e. motor adaptation, has been found to be normal in patients with CD (Sadnicka et al., 2014), hence leading to question about the role of the cerebellum in the pathophysiology of dystonia. However, patients with different types of isolated focal dystonia have been instead found to have lower rates of classical conditioning when compared with healthy controls (Teo et al., 2009; Hoffland et al., 2013; Kojovic et al., 2013). In all the former studies the presence of associated tremor has been however overlooked.

Therefore, the aim of the study at issue has been to test if a cerebellar dysfunction, studied by means of EBCC, segregates with the presence of tremor in patients with dystonia.

Materials and Methods

Population

Patients with idiopathic isolated CD were prospectively recruited among those attending the movement disorders outpatient clinic at the National Hospital for Neurology and Neurosurgery, London. All of them underwent an extensive neurological examination,

including also a video recording of patients' motor signs. Patients with known acquired or genetic forms of dystonia were excluded from the study on the basis of clinical records and examination. A great deal of effort was taken in order to characterize tremor in all the patients. To depict head tremor, patients were examined in different positions: seated upright, with the head in neutral position and while turning the head to either side. Arm tremor as well was assessed in different positions, i.e. with arms relaxed on the lap for the evaluation of rest tremor, arms outstretched or flexed at the elbow for postural tremor, and finger-to-nose maneuver for kinetic tremor. We instructed the patients to maintain each condition for 15 s. After the examination, patients were judged tremulous if tremor was present for at least 50% of each condition. Finally, patients were classified as having dystonic tremor (DT) if they showed cervical tremor with irregular amplitude and superimposed jerks, which are recognized as characteristic of dystonic tremor, while tremor affecting a non-dystonic body part was defined as tremor associated with dystonia (TAWD), as per consensus statement of the Movement Disorder Society (Deuschl, 2003). Clinical assessment included also: the scale for the assessment and rating of ataxia (SARA scale) (Schmitz-Hübsch et al., 2006), the Fahn-Tolosa-Marin Tremor Rating Scale (TRS) (Fahn et al., 1993) for the rating of tremor and the Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) (Fahn et al., 1987) for assessing the severity of CD.

All the patients were on treatment with botulinum toxin injection and therefore the study was performed at the wearing-off of the treatment, i.e. at least three months after the last injections. Patients taking additional drugs for their neurological condition were excluded from the study. We finally selected 25 patients with isolated idiopathic CD. In order to compare findings, we performed the same measurements in 12 age-matched healthy subjects (HS).

Paradigms

Blink reflex and blink reflex recovery cycles

Stimulation of the supraorbital nerve evokes an early ipsilateral response (R1) and two late responses, one ipsilateral to the stimulation (R2) and the other contralateral (R2'). The afferent limb of the reflex loop is due to the sensory trigeminal root and the ophthalmic division, whereas the efferent limb consists of the facial nerve.The R1 response is an oligosynaptic reflex response mediated through the pons. The R2 and R2' are polysynaptic reflex responses mediated through the pons and the lateral medulla and correlate with closure of the eyelid. Nerve impulses responsible for R2 are conducted by the descending spinal tract through the dorsolateral region of the pons and medulla oblongata to the lower spinal trigeminal nucleus. From there, impulses are relayed through polysynaptic medullary pathways ascending both ipsilaterally and contralaterally to the stimulus side, before making connections with the facial nuclei. The impulses cross in the lower medullary region. Trigemino-facial connections are thought to pass through the reticular formation and lie medial to the spinal trigeminal (Berardelli et al., 1999).

Stimulation of the infraorbital nerve always evokes an R2 response but not necessarily an R1. For normative value and procedure, please see Berardelli et al. (1999).

The study of the habituation, the recovery cycle and the pre-pulse modulation, use the R2 blink reflex to study the excitability of the brainstem reticular formation and the cortico-reticular drive.

The recovery cycle is studied by applying two shocks of equal intensity (conditioning and test stimuli) to the supraorbital nerve at interstimulus intervals ranging from 100 ms to 2 s. The size of the response to the test stimulus, measured as the rectified and integrated EMG activity, is

expressed as a percentage of the size of the response to the conditioning stimulus (Kimura, 1973).

Physiologically, repeated stimulation should produce habituation and decrease the amplitude and the numbers of responses. Blink reflex recovery has extensively been studied in a number of diseases, including movement disorders, cortical lesions, Bell's palsy, and brain stem lesions (Beradelli et al., 1999).

Eye blink classical conditioning (EBCC)

EBCC is a form of classical conditioning that has been widely used to study neural structures and mechanisms that underlie learning and memory. The procedure is rather simple and typically consists of pairing a conditioned stimulus (CS) (usually an auditory or visual stimulus) with an eyeblink-eliciting unconditioned stimulus (US) (e.g., a mild puff of air to the cornea or a mild shock with electrical stimulation). Physiologically this paradigm induces a reflexive, unconditioned response (UR) that follows US onset. After many CS-US pairings, an association is formed, i.e a conditioned response (CR) that precedes US onset. The magnitude of learning is related to the percentage of all paired CS-US trials.

A great body of evidence proposed the cerebellum as the essential structure for producing eyeblink CRs and particularly the interposed nucleus has been proposed as the critical site to learning, retaining, and executing the conditioning blink response (Thompson et al., 2009). Of course, structural or functional abnormalities involving the afferent or efferent branches connecting structure might influence the responses.

The US travel with the trigeminal nucleus and sends efferent projections to the inferior olive, from there the climbing fibers project to both the deep cerebellar nuclei and Purkinje cells in the cerebellar cortex. When the CS is a tone, auditory information is received via the cochlear nuclei and then from the pons the mossy fibers transmit to the cerebellum, and terminate in

both the cerebellar nuclei, and at granule cells of the cerebellar cortex. Output from the interpositus nucleus includes projections to the red nucleus, and the red nucleus sends projections to the facial and abducens nuclei. These nuclei supply the motor output component of the reflexive eyeblink (Gerwing et al., 2007).

Experimental procedures

Neurophysiological measurements included the study of the R2 blink reflex recovery cycle (BRC) and of the EBCC.

The BRC and the EBCC were recorded in all subjects according to standard methods (Teo et al., 2009).

To test the BRC, square wave pulses of 200μs width at 5 times the somatosensory threshold (ST) were used to stimulate the right supraorbital nerve. Single or double pulses were given randomly at interstimulus intervals (ISI) of 200, 300, 400 and 1000 ms. A total of six trials for each ISI were collected. Surface electromyographic (EMG) activity was recorded from the right and left orbicularis oculi muscles. After the recordings, the signal was analyzed off-line and the recorded activity was DC-corrected, rectified, and averaged. After the measurements, the ratio between unconditioned and conditioned R2 area was calculated.

In order to collect EBCC, we used conditioning stimulus (CS), a loud (70-80 dB; 2000 Hz) tone lasting 400 ms, which was delivered by means of binaural headphones. The CS inconsistently produced an acoustic startle response ("alpha blink") occurring within 200 ms after the CS. The unconditioned stimulus (US) was instead a square electrical pulse of 200 μs length and of intensity equal to five times the ST, which was delivered over the right supraorbital nerve 400 ms after the CS. Surface EMG was recorded bilaterally from the orbicularis oculi muscle. Pairs of CS and US at 400 ms ISI were delivered in 6 acquisition blocks (each consisting of 9 CS-US

pairs, 1 US only, 1 CS only trial). A seventh block consisted of 11 CS-only trials to measure extinction.

EMG bursts were considered “alpha blinks” if their amplitude exceeded 50 μV and if they occurred within 200 ms after the CS. In CS-US pairs, EMG bursts were considered instead conditioned responses (CRs) if latency was at least 200 ms after the CS but before the US. For trials including CS only, EMG bursts occurring 200–600 ms after the CS were considered CRs.

Statistical analysis

Clinical differences between patients with and without dystonia were assessed with several Mann-Whitney tests, while a one-way ANOVA was used to compare the intensity for evoking the R2 component of the blink reflex in all groups.

Mixed ANOVAs with “group” (dystonia without tremor, dystonia with tremor, HS) and “ISI” (200, 300, 400 and 1000 ms) as factors of analysis were used to assess differences in R2 recovery.

Several Kruskal-Wallis tests were instead used in order to compare the number of CRs summed over all blocks and in each block (from 1 to 7) in the three groups. Finally, we applied Mann-Whitney tests in order to calculate differences among groups (dystonia without tremor, dystonia with tremor and HS) in significant blocks.

Possible correlations among demographic data, clinical features (disease duration, TRS and TWSTRS) and neurophysiological results in the two groups of patients were evaluated with the Spearman’s rank correlation coefficient.

When using ANOVAs, normal distribution of data was assessed by means of Shapiro-Wilks’ test and Levene’s test was used to assess homogeneity of variance across groups. Greenhouse Geisser correction was used when necessary to correct for non-sphericity (i.e., Mauchly's test <

0.05). All p values < 0.05 were considered significant. Bonferroni post-hoc test was used for post-hoc analyses following the ANOVA. Bonferroni correction was used to correct for multiple comparisons.

Results

The 25 patients with isolated idiopathic CD were categorized as with tremor (n=13) and without tremor (n=12) depending on the results of clinical examination and on the review of the videotapes by two experts in movement disorders.

Among patients with tremor, 8 had DT and 5 had both DT and TAWD.

Statistical analysis did not disclose significant clinical differences among patients with and without tremor **(Table 1)**.

Table 1: Clinical features of dystonic patients.

Patients with Dystonia					**Patients with Dystonia and Tremor**							
Case	**Sex**	**Age**	**Disease duration**	**TWSTRS**	**Case**	**Sex**	**Age**	**Disease duration**	**TWSTRS**	**DT**	**TAWD**	**TRS**
1	F	65	20	14	**1**	M	68	15	15	X	hands	12
2	F	68	13	16	**2**	F	60	14	23	X	hands	9
3	M	48	14	16	**3**	F	65	15	9	X		10
4	F	36	6	9	**4**	M	55	15	14	X		5
5	F	58	10	14	**5**	F	70	15	16	X		7
6	M	64	12	29	**6**	F	74	22	22	X	hands	12
7	F	76	14	18	**7**	F	42	16	20	X		4
8	F	54	6	15	**8**	M	52	12	18	X		6
9	F	66	31	41	**9**	F	54	26	22	X	hands	20
10	F	64	11	30	**10**	M	68	8	40	X	hands	16
11	F	64	11	20	**11**	F	58	16	14	X		6
12	M	49	16	15	**12**	F	69	20	21	X		5
					13	M	72	30	22	X		5
Av		**59.3**	**13.7**	**19.7**	**Av**		**62.1**	**17.2**	**19.7**			**9.0**
SD		**10.8**	**6.7**	**9.05**	**SD**		**9.45**	**5.89**	**7.41**			**4.86**

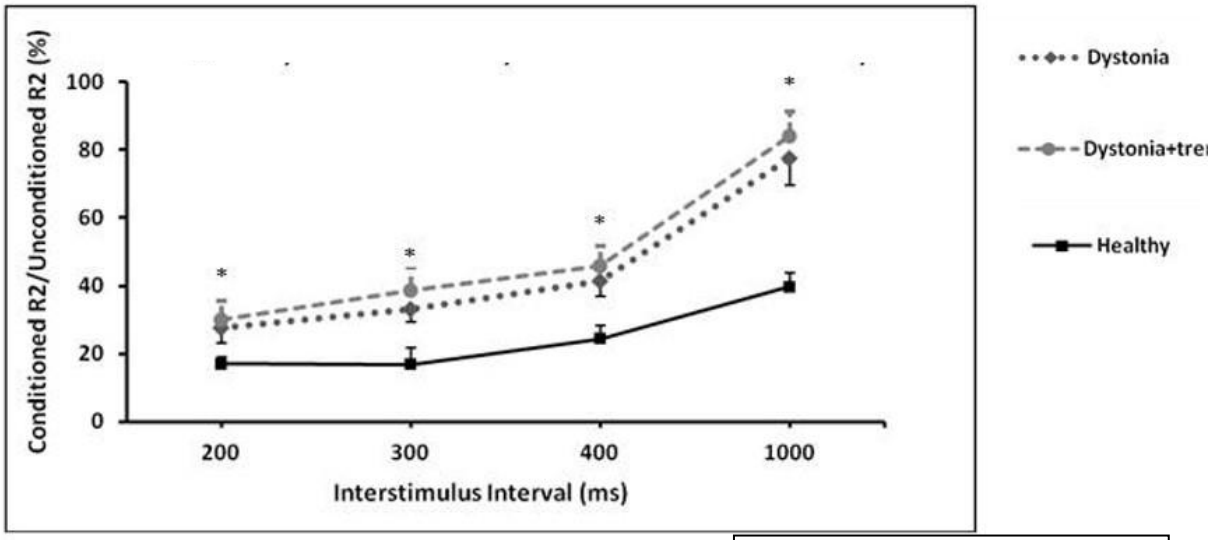
Figure Legend: Age and disease duration are measured in years. Av: average; DT: dystonic tremor; F: female; M: male; SD: standard deviation; TAWD: tremor associated with dystonia; TWSTRS: Toronto western spasmodic torticollis rating scale; TRS: Fahn-Tolosa-Marin tremor rating scale; X: means presence of dystonic tremor.

Blink reflex excitability

Unpaired t-tests displayed comparable intensity (dystonia without tremor: 6.46±2.23 mA; dystonia with tremor: 6.72±3.47 mA; HS: 6.4±2.30 mA) in eliciting blink reflex in all groups. The mixed ANOVA disclosed a significant effect of "group" ($F_{2,34}$= 12.35; P<0.001),"ISI"($F_{1.5,52.4}$=51.54; P<0.001) and a significant interaction of "group×ISI" ($F_{3.1,52.37}$=2.53; P=0.025).

Post-hoc analyses showed that patients with and without tremor did not differ in any of the ISI considered, while there was a significant effect at all ISI when comparing both groups of patients with HS (**Figure 1**).

Figure 1



Modified from Antelmi et al, PRD 2016

Blink recovery cycle in healthy subjects and patients with dystonia (with and without tremor). Asterisks represent significant results (p < 0.05) when comparing blink recovery cycle curves of

the R2 component in patients and in healthy subjects. Error bars indicate the standard error. ISI: interstimulus interval.

Eyeblink classical conditioning

Kruskal-Wallis test showed a significant difference in CRs summed over all blocks among the three groups (H2=8.49, P=0.014).

Mann-Whitney tests showed that HS and patients with dystonia without tremor had the same total number of CRs, while patients with dystonia and tremor had reduced number of CRs compared both with HS (Z=-2.96, P<0.05) and with patients with dystonia without tremor (Z=1.97, P<0.05).

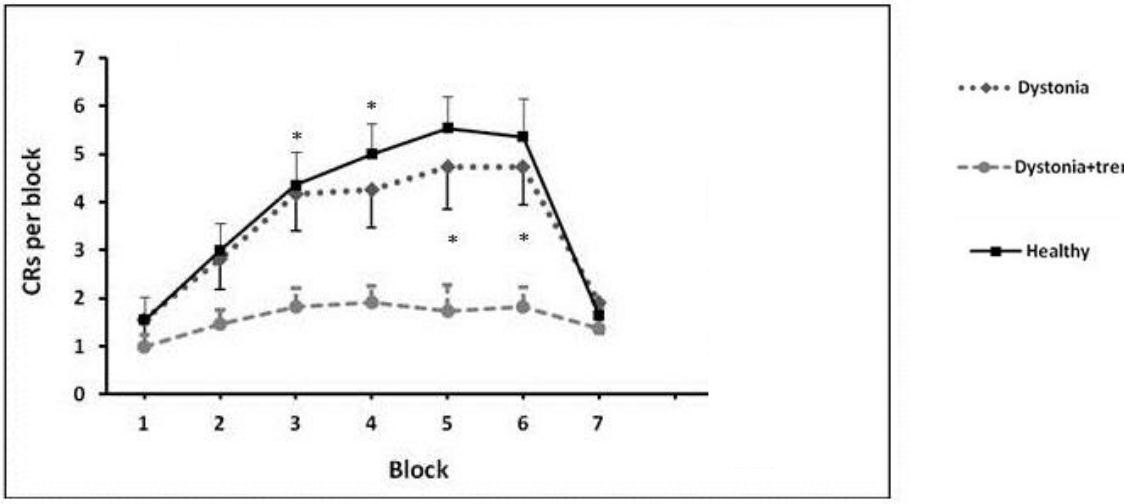
Considering the rate of CRs over different blocks, Kruskal-Wallis tests showed a significant difference among the three groups in Block 3 (H_2=6.92, P=0.028), Block 4 (H_2=9.19, P=0.007), Block 5 (H_2=11.35, P=0.002) and Block 6 (H_2=8.42, P=0.012).

Mann-Whitney tests showed that patients without tremor and HS did not differ with regard to CRs in all blocks, while patients with tremor and HS differed in Block 3 (Z=-2.87, P=0.003), Block 4 (Z=-3.27, P=0.001), Block 5 (Z=-3.32, P<0.001) and Block 6 (Z=-2.93, P=0.002).

Regarding patients with tremor, Mann-Whitney tests did not show a significant difference in CRs in all blocks considered between patients with DT and patients with both DT and TAWD.

Finally, patients without tremor and patients with tremor differed in conditioning in Block 5 (Z=-2.36, P=0.016) and Block 6 (Z=-2.01, P=0.045). (**Figure 2**).

Figure 2



Modified from Antelmi et al, PRD 2016

Eyeblink classical conditioning in healthy subjects and patients with dystonia (with and without tremor). Asterisks represent significant results ($p < 0.05$) when comparing the number of conditioned eyeblink responses per block in tremulous patients and in healthy subjects (line above) and in patients with tremor and patients without tremor (line below). Error bars indicate the standard error. CRs: conditioned responses

Correlation of BRC and EBCC with clinical features

Spearman's rank correlation coefficient did not disclose any correlation between neurophysiological results and clinical data (disease duration, TRS and TWSTRS).

Discussion

In our cohort of CD patients we found that impaired cerebellum functioning, tested by means of EBCC paradigm, segregates with the presence of tremor in patients with CD. Indeed, results showed that patients with tremor had lower rates of CRs when compared to both HC and non-tremulous patients.

The EBCC is a well-known paradigm of associative learning, and it has been largely demonstrated that it is strongly cerebellar dependent (Gerwig et al., 2007). There is indeed a

great bulk of literature on animals showing that cerebellum and its associated circuitry constitute the entire essential circuit for delaying classical conditioning of eyeblink and other discrete responses (Thompson and Steinmetz, 2009). Anatomical and neurophysiological data, thoroughly support the hypothesis that the essential memory trace for the learning of these discrete conditioned responses is formed and stored in the cerebellar interpositus nucleus (Thompson and Steinmetz, 2009). Neuronal/synaptic plasticity is also established in the cerebellar cortex, but its role is less clear as well as the role of the hippocampus (Moyer et al., 2015). There is some evidence that EBCC may be also influenced by the brainstem reflex centres of the eyeblink reflex, via the red nucleus (Bacha, 2004), along with the pontine nuclei and the inferior olive. However, in our population of dystonic patients, it is unlikely that brainstem excitability accounts for the differences between dystonic patients with and without tremor, as both groups of patients showed a similar degree of brainstem hyperexcitability, as measured by means of the BRR. Therefore, abnormal brainstem inhibition cannot be claimed as the cause of decreased conditioned responses in the tremulous group.

On this basis, a specific role of the cerebellum in the development of tremor in patients with dystonia may be supported.

The cerebellar hypothesis in dystonia has increasingly gained huge momentum based on a body of clinical, neurophysiological and imaging evidence (Prudente et al., 2014). In dystonic patients contrasting results have been obtained with regards to the EBCC. In fact, the EBCC has been reported to be impaired in different types of primary focal dystonia (Teo et al., 2009), but not in patients with hereditary dystonia due to *TOR1A* or *THAP1* mutations (Sadnicka et al., 2015) or in secondary dystonia (Kojovic et al., 2013). Such a discrepancy might be due to the fact that different pathophysiological mechanisms might be responsible for different types of dystonic syndromes. Indeed, the occurrence of tremor in early onset dystonia seems to be

lower, while tremor is more frequently reported in late onset idiopathic dystonia, and particular in segmental (craniocervical) and CD (De Fazio et al., 2015). Moreover, the contradictory results reported so far might also have been influenced by the the fact that the presence of tremor has been overlooked in previous cohorts.

Overall, the cerebello-thalamo-cortical pathway is generally reckoned to be implicated in the pathophysiology of tremor and indeed decreased conditions at the EBCC paradigms have been reported also in other types of tremor, including ET (Kronenbuerger et al., 2007) and neuropathic tremor from acquired disease (Schwingenschuh et al., 2013).

As reported above, abnormal oscillatory activity in the internal globus pallidus (GPi) (Liu et al., 2008) has been claimed as a possible mechanism of tremor in dystonia. However, while DBS of the GPi ameliorates dystonic symptoms, it does not always have such a good impact on the tremulous component. This might suggest that other structures beyond the GPi account for the occurrence of tremor in dystonia. Indeed, as a further proof, thalamic DBS, particularly of the ventral intermediate nucleus (e.g., the main target of cerebellar projections to the thalamus) improves tremor in dystonic patients (Pauls et al., 2014) and stimulation applied to Vim (the main target of cerebellar projections to the thalamus) have been reported to improve tremor in dystonia (Hedera et al., 2013). In this regard, anatomopathological abnormalities involving the cerebellum have been found in dystonic patients with additional tremor (Ma et al., 2012) and, recently, a study of functional MRI showed s discrete involvement of the cerebellum in patients with dystonic tremor vhen compared to dystonia (Kirke et al, 2016). Hence our results together with the above reported body of evidence would further suggest that the development of tremor in patients with dystonic syndromes involves the cerebellum. Clinical examination in our patient cannot disclose instead any clear cerebellar sign. This is in line with what has been previously reported in literature, as recently reviewed by Prudente et al. (2014).

The absence of clear clinical cerebellar signs in dystonic patients points toward a selective impairment of an isolated pattern of motor control linked to the cerebellum. Paradigms of non-invasive cerebellar stimulation in patients with dystonia (Hoffland et al., 2013) have been proved to ameliorate conditioning responses. This might suggest that cerebellar dysfunctions in dystonia might be dynamic, occurring at a functional rather than at a structural level.

In keeping with the model suggesting dystonia as a network disorder, our study points towards a functional cerebellar impairment as major determinant for the occurrence of tremor in patients with dystonia.

The results of our study give new insight into the pathophysiology of tremor in dystonia, claiming cerebellum as a crucial node underlying the pathophysiology of tremor in dystonia. Moreover, these findings might be important also for their potential treatment implications. Indeed, on the basis of these results, it is possible to infer that tremor might benefit from chemical, functional or surgical approaches targeting not only the basal ganglia, but also the cerebellum.

Chapter 3

SECOND STUDY

Neurophysiological correlates of abnormal somatosensory temporal discrimination in dystonia.*

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Background

Formerly considered a pure basal ganglia-motor disorder, dystonia is currently recognized as a disorder of the sensorimotor network. However, as reported in the general introduction, the pathophysiology of dystonia is still a puzzling issue.

Impaired inhibition rises as the leading functional abnormality (Hallet and Rothwell, 2011), causing derangement of plasticity (Quartarone et al., 2011). Clinical observation (overflow dystonia, mirror dystonia, presence of sensory trick), behavioral and neurophysiological measurements (Hallet and Rothwell, 2011, Quartarone et al., 2006; Tinazzi et al., 2000) all point to the same direction, suggesting a derangement of homeostatic plasticity (Quartarone and Pisani, 2011). Abnormal inhibition has been found both in the somatosensory (S1) and in the motor (M1) cortex. However, the ultimate neuronal substrate underlying such abnormalities is still debated. Impairment of lateral inhibition within the S1 (Angel, 1967; Shagass and Schwartz 1964; Wiederholt, 1978) may explain the abnormalities reported when analyzing somato-sensory potentials by coupled stimulations (Frasson et al., 2001; Tamura et al., 2008) or by simultaneous stimulation of two different sites (Tinazzi et al., 2000). However, whether they are due to abnormal functioning of local inhibitory interneurons within the S1 (Tamura et al., 2008) or to an abnormal modulation by the thalamo-cortical afferents (Frasson et al., 2001) is still debated. Sensory abnormalities in dystonia have been also reported in the spatial domain and abnormal sensory processing of dual inputs, such as a putative marker of impaired lateral surrounding inhibition, has been found in generalized dystonia (Tinazzi et al., 2000).

The temporal domain is affected as well and particularly somatosensory temporal discrimination (STDT) has been consistently reported to be abnormal in patients with different

types of dystonia (Molloy et al., 2003; Fiorio et al., 2003, 2008; Tamura et al., 2008; Scontrini et al., 2009).

STDT is defined as the minimum interval between two pairs of tactile stimuli to be clearly perceived as separate. Healthy individuals usually perceive two tactile stimuli as sequential when the interstimulus interval (ISI) exceeds 30–50 msec (Lucruz et al., 1991), although there is inter-subject variability and a progressive decline of performance with age (Kimmich et al., 2014; Ramos et al., 2016). Different brain regions have been claimed to be implicated in STDT, including the primary somatosensory area (S1), the pre-supplementary motor area, and the basal ganglia in both healthy subjects (Kimmich et al., 2014; Ramos et al., 2016) and in patients with focal cerebral lesions (Lacruz et al., 1991). In dystonia, impaired STDT does not correlate with disease severity (Molloy et al., 2003; Conte et al., 2012), it is abnormal in non-dystonic body regions (Fiorio et al., 2003), but also in about half of unaffected first-degree relatives of patients (Fiorio et al., 2008; Tamura et al., 2008). All these findings lead to consider STDT as an endophenotypic trait of dystonia. So far, few studies have instead focused on the neurophysiological substrates of abnormal STDT in dystonia. The study by Tamura et al. (2008) addressed this iusse by measuring paired pulse (PP) somatosensory evoked potential (SSEP) recovery cycle in patients with focal hand dystonia and impaired STDT. The authors reported reduced PP SEEP recovery cycle of the P27 component at the short interstimulus interval (ISI) of 5 ms, a measurement thought to be linked to altered S1 intracortical inhibition, to be linked with abnormal STDT in focal hand dystonia. STDT has been reported to be related to inhibitory mechanisms within S1 also in healthy subjects (Pastor et al., 2004). In this regard, additional indirect suggestions come also from studies assessing modulation of STDT by paradigms modulating synaptic plasticity within the S1, in both healthy subjects and patients with focal dystonia (Conte et al., 2014; Erro et al., 2015). Studies of neuroimaging have instead correlated

abnormal STDT in dystonia with other regions of the sensorimotor network, including the cerebellum and the basal ganglia (Tinazzi et al., 1999; Sanger et al., 2001; Scontrini et al., 2009), suggesting an abnormal functioning of a subcortical striatal re-entrant looped pathway. Thus, the relative contribution of each of the nodes of the sensorimotor network to abnormal temporal discrimination in dystonia is not entirely clear.

Conte and colleagues (2014) reported that in both patients with focal hand dystonia as well as in healthy subjects, STDT was improved by a protocol of intermittent theta burst stimulation, which is supposed to induce synaptic plasticity within S1 by long-term potentiation mechanisms. Similarly, Erro et al. (2015) found SDTD to be ameliorated in healthy subjects by high frequency sensory stimulation of the skin, and hence by a mechanism likely linked to plastic changes within the S1. However, none of these studies evaluate measurements of cortical inhibition directly.

A simple way to look at the activity of inhibitory neurons within the S1 is by studying the high frequency oscillations (HFOs). HFOs indeed measures the activity of inhibitory mechanism within the somatosensory system. Particularly, the “early” HFO burst is tought to be generated from action potentials of thalamocortical fibers at the time when they arrive at the primary somatosensory cortex, while the “late” component seems to be generated by intracortical inhibitory interneurons (Ozaki and Hashimoto, 2011). HFOs have been once studied in patients with focal dystonia and found to be abnormal (Inoue et al., 2004).

In order to evaluate how mechanisms of sensory inhibition at different levels of the somatosensory system may contribute to abnormal temporal processing in dystonia, we have here performed an extensive neurophysiological battery in patients with isolated idiopathic CD.

We therefore tested the cortical levels by measuring PP SSEP at short inter stimulus interval, double SSEP by simultaneous stimulation of two different fingers nerves and late component of HFOs and at subcortical levels by measuring recovery cycle of SSEP at longer interval and early component of the HFOs, with the hypothesis that decreased inhibition at cortical level correlates with abnormal STDT.

Materials and Methods

Participants

We prospectively recruited patients with idiopathic isolated CD, according to current criteria (Albanese et al., 2013) from those attending the outpatient clinic at the Sobell Department of Motor Neuroscience and Movement Disorder, Institute of Neurology, UCL, London, UK. All the patients were on treatment with botulinum toxin injection, but no additional medications. Patients were assessed at the wearing-off of the treatment and therefore at least 3 months after their last botulinum toxin injection. All underwent an extensive clinical examination, and clinical records were carefully reviewed in order to exclude other forms of dystonia. Disease severity was assessed with the Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS). We finally recruited nineteen patients. The study included also nineteen age- and sex-matched healthy volunteers. In all the HC we collected a clinical history and performed a neurological examination, finally excluding all subjects with a possible family history of any neurological disorders, including dystonia. 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 central nervous system and 3) no symptoms/signs suggestive of a peripheral neuropathy.

All experimental procedures were approved by the local institutional review board and conducted in accordance with the Declaration of Helsinki and according to international safety guidelines.

Paradigms

Somatosensory Temporal Discrimination Threshold (STDT)

Temporal discrimination is a basic aspect of somatosensory processing, critical for a number of sensory functions including kinesthesia, graphesthesia, vibratory sense and stereognosis. STDT is the shortest time interval at which two separate stimuli are perceived as asynchronous. In healthy subjects, it ranges from 30 to 40 ms (Hoshiyama et al., 2004) and it increases with age. Strunctural and functional studies highlighted that temporal discrimination of tactile stimuli is a complex task requiring the involvement of several cortical and subcortical areas, such as pre-supplmentary motor area, anterior cingulate cortex, and basal ganglia, along the primary sensory areas (Pastor et al., 2004).

Sensory Evoked Potentials (SEPs)

SEPs are electrophysiological responses (potentials) to the stimulation generated in the sensory pathways at several (peripheral, spinal, subcortical and cortical) levels of the nervous system. By means of SEPs it is possible to assess the function of the dorsal column–lemniscal system from the first-order neurons (dorsal root ganglia) to the fourth order neurons (somatosensory cortex areas). They are typically evoked by bipolar transcutaneous electrical stimulation applied on the skin over the selected nerve. These electrical stimuli depolarize nerve fibers directly by generating a potential difference in the medium adjacent to the nerve trunk and across the nerve fiber membrane, causing depolarization close to the site of cathode. Cathode should be proximal to the anode in order to prevent anode block. The

selected nerves are stimulated with monophasic square wave electrical pulses and stimuli are delivered by using either a constant voltage or a constant current stimulator. The stimulus intensity is set slightly above the motor threshold for mixed sensory-motor nerves and at three or four times the sensory threshold for stimulation of sensory nerves. A rate of 1-10 stimuli per second (1-10 Hz) is usually used. Signal averaging reduces noise that is random with respect to stimulus delivery while retaining signals that are time-locked to stimulus delivery. SEPs typically are recorded by using standard EEG electrodes. Recording electrode sites are identified by anatomical landmarks, according to the international 10-20 system, or its extension, the 10-10 system. To minimize the electrode artifact the ground should be placed on the stimulated limb, between the site and the recording electrode. However, electrically isolated stimulators allow the use of the ground electrode on the head, which is adequate to eliminating artifact related to the electrical main and radiofrequency interference. The contact impedances of the stimulating electrodes should be kept low (Cruccu et al., 2008), below 5,000 ohms, and should be as uniform as possible across the electrodes to maximize common-mode rejection and minimize noise pickup. Most of the SEP components peak before 50 and 100 ms.

Typical recording amplifier filter settings for SEPs are 30-3,000 Hz. SEPs are composed of both low and high frequencies, and filtering can be problematic.

The number of sweeps that need to be averaged depends upon the initial signal-to-noise ratio, but generally, 500 to 1000 sweeps are enough. There are many factors that may influence SEP amplitude and latency (namely age, body height and gender, skin and core temperature, attention and vigilance, drugs).

Several characteristics of SEPs can be measured, including latency, peak latencies, component amplitudes, and waveform morphology. SEP components typically are named by their polarity and typical peak latency in the normal population. Different components may be recognized. In

our experimental procedure we focused on brainstem and cortical component of SEPs, i.e. P14, which is the potential arising from the lower brainstem close to the cervico-medullary junction and N20 which is localized to the parietal scalp region, showing a polarity reversal across the central fissure. It represents the largest early negative deflection at parietal scalp region. It is generated from the primary somatosensory cortex in the posterior wall of the central fissure.

High Frequency Oscillations (HFOs)

HFOs are fast oscillations (at around 600 Hz) that have been firstly described in healthy subjects underlying the N20 component of SEPs (Cracco and Cracco, 1976). They have been claimed to represent the cortical activity of the GABAergic inhibitory interneurons monosinaptically receiving thalamocortical inputs (Ozaki and Hashimoto, 2011). They can be separated from N20 applying a digital band-pass filtering (500-1000 Hz). The early HFO burst has been reported to be generated from action potentials of thalamocortical fibers at the time when they arrive at the primary somatosensory cortex, while the late component seems to be generated by local inhibitory interneurons. Indeed, the later part of HFOs, has been found to be sensitive to stimulus rate, reflecting therefore the activity of postsynaptic neural network in the areas 3b and 1, whereas the early part of them may result from action of thalamocortical fibers, which are usually resistant to frequency stimulation (Urasaki et al., 2002). N20 and Late HFOs show a reciprocal relationship. Indeed, factors that modify the one have an inverse influence on the other. Protocols of brain manipulation showed that HFOs and SICI showed a parallel response to stimulation (Murakami et al., 2008a) and it has been speculated that a common neural mechanism is involved in the generation of SICI in the motor cortex and Late HFOs in the somatosensory cortex, i.e., the activity of GABAergic interneurons and their networks with pyramidal cells.

Paired stimulation SSEP (PP SSEP)

PP SSEP might be used to study surround inhibition. Indeed, physiologically, a conditioning (preceding) stimulus induces a suppression of SSEP amplitudes evoked by following test stimulus (Angel et al., 1967) in order to preserve the temporal separation of serially administered stimuli.

Experimental procedure

SSEPs recording and analysis

We evaluated P14 and N20 SSEPs amplitude and latency, SSEPs recovery cycle and spatial inhibition ratio (SIR) (**Figure 3**). SSEPs were recorded from scalp Ag–AgCl surface electrodes arranged according to the international 10-20 system of EEG electrode placement. To record 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 right thumb (T) and index (I) fingers 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 the first block, 1000 sweeps were averaged; N20 peak latency and N20-P25 peak to peak amplitude were measured. The recording from this block was also used to extract and measure HFO, as explained in the next paragraph. Three more recording blocks of 750 frames each were performed to measure N20-P25 recovery cycle. In each of them, 750 trials were averaged and paired pulses at ISI of 5, 20 and 40 ms were delivered (Meyer-Hardting et al., 1983; Vollono et

al., 2010). In the frames obtained using paired stimuli, the responses following the second stimulus were obtained by subtracting the SSEPs waveform obtained by the first stimulus from the waveform following each double stimulus (Meyer-Hardting et al., 1983; Vollono et al., 2010). R5, R20 and R40 were defined as the ratio between the second and the first response (Meyer-Hardting et al., 1983; Vollono et al., 2010). Finally two more blocks of 750 trials each were recorded, the first one stimulating the right thumb only and the second one stimulating both the thumb and index finger concomitantly by giving two simultaneous stimuli delivered through two constant current stimulators. The spatial inhibition ratio (SIR) of N20-25 and P14 was calculated as the ratio TI/(T+I)x100, where TI is the SSEP amplitude obtained by simultaneous stimulation of the thumb and index finger and T+I is the arithmetic sum of the SEP obtained by the individual stimulation of the two fingers (Tinazzi et al., 2000). In healthy subjects, spinal brainstem and cortical SSEPs to simultaneous dual inputs are expected to be smaller than the sum of each alone because of lateral inhibition between the two inputs (Tinazzi et al., 2000).

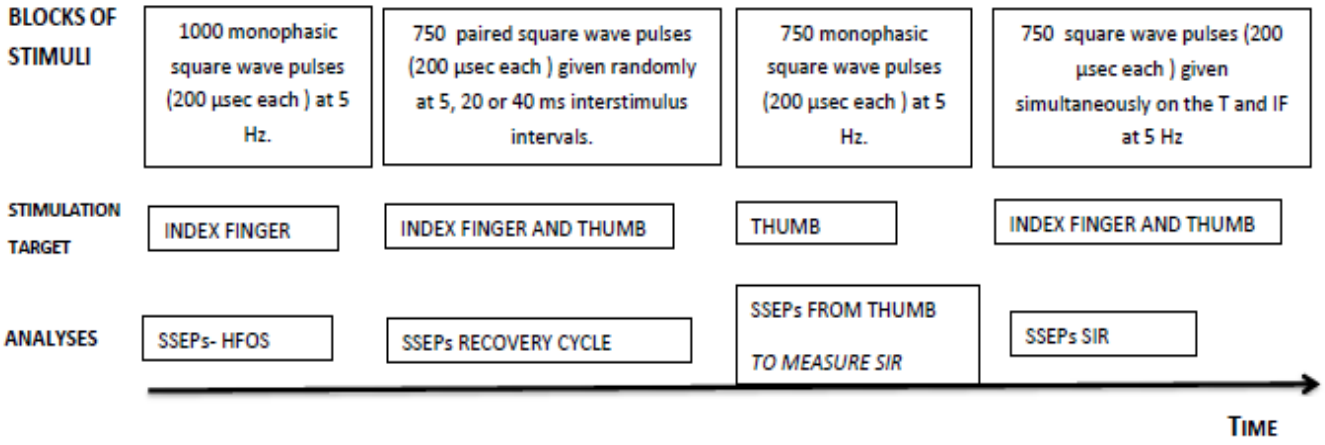
When we performed all the neurophysiological tests, we tried to ensure that both patients and HC were seated comfortably and quietly in order to avoid the occurrence of involuntary movements, as it is well known that movement gates sensory access to cortex (Jones and Burton, 1976; Murase, 2000).

Analysis of High Frequency Oscillations

To extract HFO from the underlying N20, the stimulus artifact was removed manually from -10 to + 5 ms to avoid ringing (Katayama et al., 2010). The SSEP wide-band signal was bandpass filtered digitally (400–800 Hz) and averaged. HFO waveform was divided into two components, namely early (e-HFO) and late (l-HFO), separated by the latency of the N20 peak. Onset of e-HFO and offset of l-HFO were defined as the time point at which their amplitudes exceeded the

averaged background noise level by 3 SDs (Murakami et al., 2008). The signal was then corrected for DC shift and rectified. e-HFO and l-HFO area under the curve were measured and analyzed.

Figure 3: Somatosensory Evoked Potentials procedure



HFO: high frequency oscillations; Hz: hertz; ms: milliseconds; µs: microseconds; SSEPs: somatosensory evoked potentials; SIR: surround inhibition ratio

Statistical analysis

Some of the gathered variables did not distribute normally and therefore non-parametric 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 patients and HC. Correlations between the variables were evaluated with Spearman's 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. All statistical analyses were performed using Stata (v. 11, Stata Corp, USA).

Results

Table 2 summarizes the demographic, clinical, behavioral and electrophysiological findings in patients and HC.

Table 2. Summary of clinical and electrophysiological features in patients and healthy controls. Significant differences are expressed in bold.

	Healthy Controls	**Patients**	**p**
CLINICAL FEATURES			
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	-
RESULTS OF THE NEUROPHYSIOLOGICAL INVESTIGATIONS			
STDT (ms): **mean values** **range**	 80.1±29.9 23.3-116.7 ms	 100.1±25.3 53.3-146.7 ms	 **0.03**
SSEP latency (ms): - **N20 thumb** - **N20 index** - **P14 thumb**	 22.35±0.9 22.96±0.9 16.33±0.6	 22.71±1.1 22.49±1.1 16.41±0.6	 0.16 0.12 0.54

- **P14 index**	16.48±0.6	16.53±0.6	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

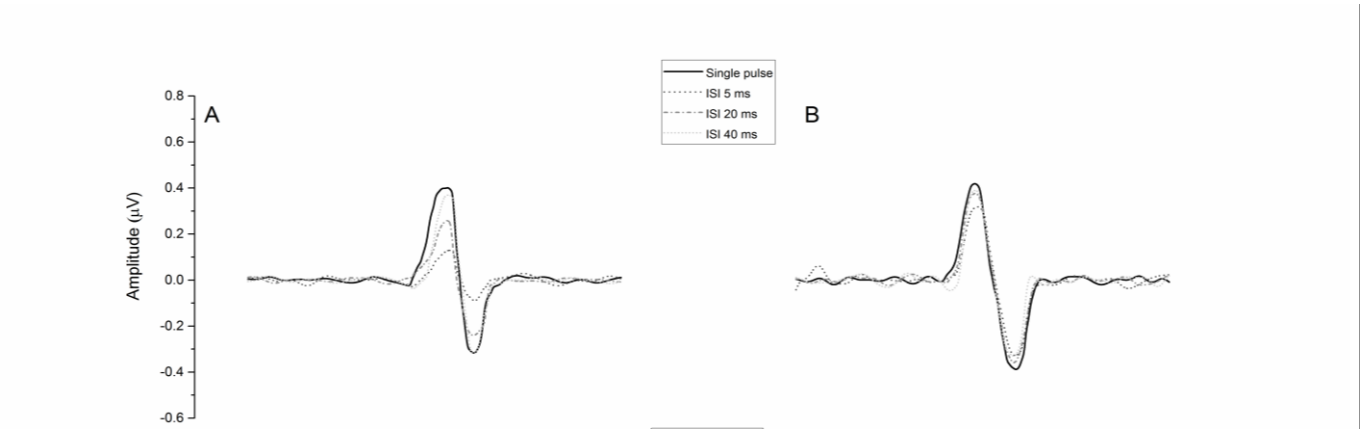
Modified from Antelmi et al, MDJ 2017

F: female; M: male; R: right; L: HFO: high frequency oscillations; ms: milliseconds; µV: microvolts; R: recovery cycle; SSEPs: somatosensory evoked potentials; SIR: spatial inhibition ratio; TWSTRS: Toronto Western Spasmodic Torticollis Rating Scale.

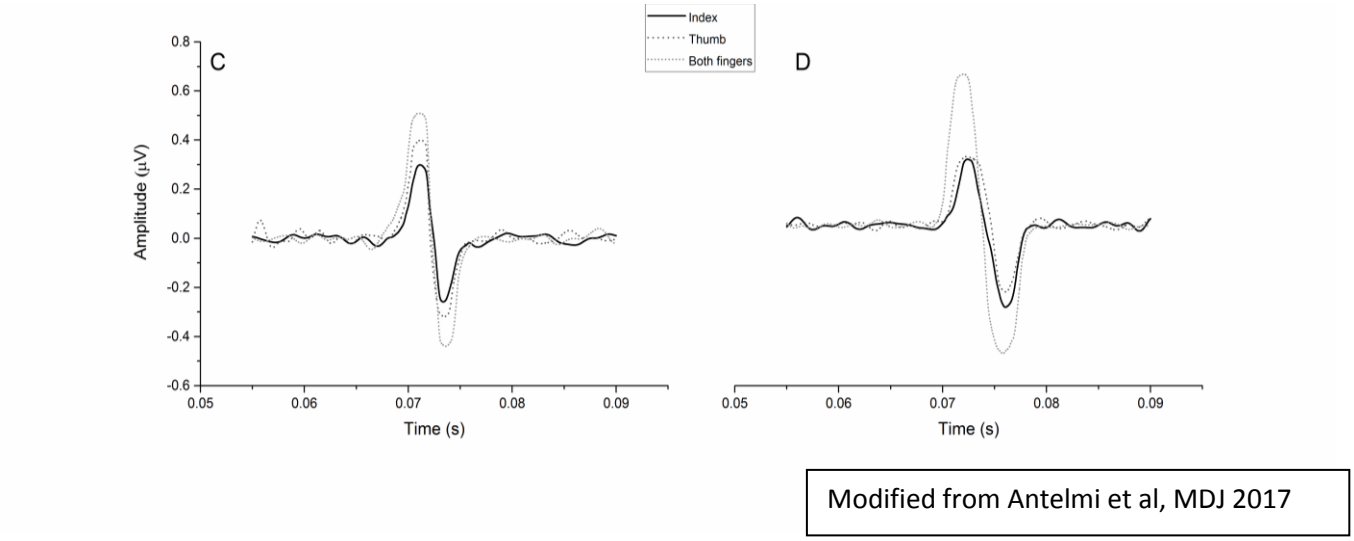
STDT was significantly higher in patients than HC (100.1±25.3 vs 80.1±29.9 respectively, p=0.03). Many of the sensory electrophysiological measures of temporal inhibition were also abnormal in the patients. Compared with the HC, paired pulse SSEP data showed reduced P14 suppression at ISIs of 5 and 40ms, while N20 suppression was reduced at all ISIs (e.g., 5, 20 and 40 ms). Electrophysiological measures of spatial inhibition following simultaneous stimulation from 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 (**Figure 4**).

Figure 4

Example of paired pulse SSEPs (upper row) and SIR (lower row) measured on the N20 wave in one healthy subject (panels A and B).



and in a patient with dystonia (panels C and D).



Modified from Antelmi et al, MDJ 2017

ISI: interstimulus interval; µV: microvolts; ms: milliseconds; s: seconds; SIR: surround inhibition ratio

Compared with the healthy subject, SSEPs recorded from the dystonic patient show less paired-pulse inhibition at all ISI and less suppression when the thumb and index finger were stimulated at the same time. The signals were bandpassed between 20 and 500 Hz for visualization purposes.

E-HFO area was smaller in patients than HC, while there was a non-significant tendency for l-HFO to be smaller in patients.

In both HC and patients, there was a strong correlation between STDT and N20 suppression at an ISI of 5 ms (Spearman's rho: 0.73, p=0.001 and 0.80, p<0.001, HC and patients, respectively) and between STDT and l-HFO area (Spearman's rho: -0.73, p=0.001 and -0.78, p<0.001, HC and patients, respectively). In addition, N20 suppression at an ISI of 5 ms was correlated with l-HFO area (Spearman's rho: -0.84 and -0.81, HC and patients, respectively, both p<0.001) (**Figure 5A and 5B**).

Figure 5 A

Correlations between STDT and SSEPs suppression at ISI of 5 ms (upper panel) and l-HFO (down panel) in healthy controls and patients.

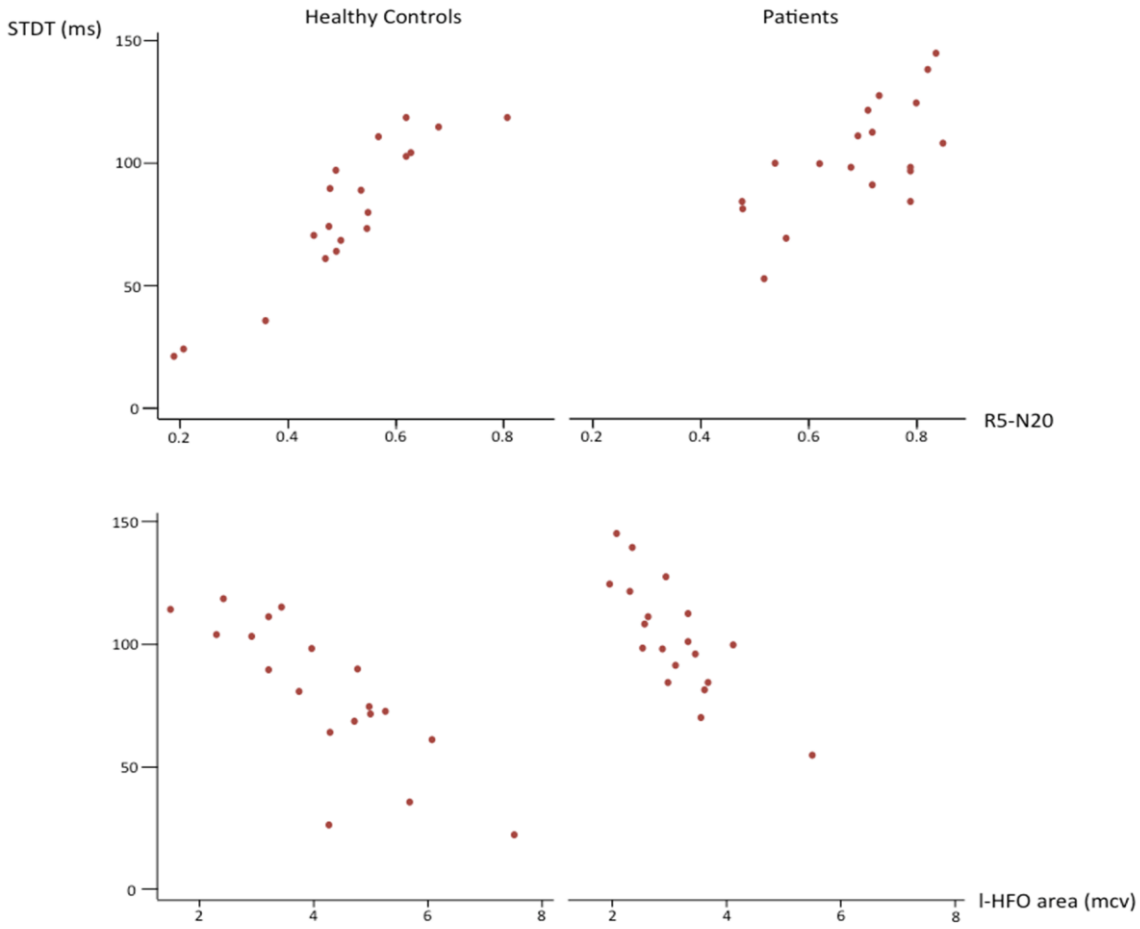
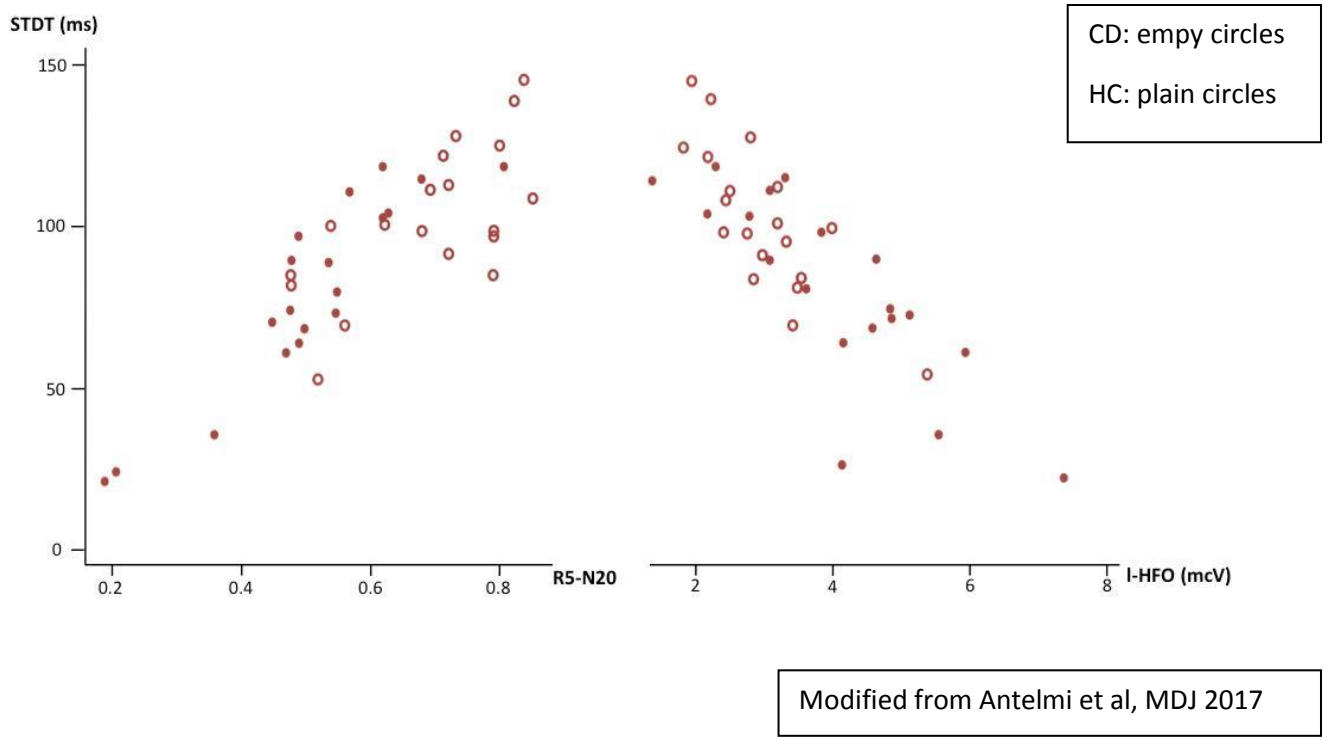


Figure 5 B

Merged correlations between STDT and SSEPs suppression at ISI of 5 ms (left panel) and l-HFO (right panel) in healthy controls (plain dots) and patients (empy dots).



STDT (somatosensory temporal discrimination threshold); l-HFO (late high frequency oscillations); R5-N20: recovery cycle at 5 ms interstimulus interval for the N20 component

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.

Finally, the logistic regression model showed that reduced N20 suppression at an ISI of 5 ms (coeff.: 67.33; $p<0.01$), smaller l-HFO area (coeff.: -11.05; $p<0.01$), and (dystonia) group (coeff.: 9.62; $p<0.05$), were independently associated with higher STDT, explaining a variance of 64%

(R-squared=0.64) (**Figure 5A and 5B**). The Hosmer–Lemeshow goodness-of-fit test supported our regression model as being valid.

Discussion

In line with previous studies (Tinazzi 1999; Sanger et al., 2001; Molloy et al., 2003; Fiorio et al.; 2003; Fiorio et al., 2008, Tamura et al., 2008; Scontrini et al., 2009; Scontrini et al., 2010; Kimmich et al., 2014), dystonic patients displayed higher SDTD values when compared to HC. Moreover, as previously reported (Fiorio et al., 2007; Scontrini et al., 2009), abnormally increased STDT values were found also in body regions not affected with dystonia and did not correlate with dystonia severity, further supporting the notion that abnormal temporal processing in dystonia is not simply a consequence of the overt manifestations of dystonia.

Our mean STDT values in both HC and patients were slightly higher than those previously reported in literature (Hoshiyama et al., 2003; Fiorio et al., 2007; Fiorio et al., 2008). Giersch et al., (2009) demonstrated that TDTs obtained using different protocols/equipments are only comparable within each individual experimental paradigm. Different procedures have been indeed used in different studies (e.g., ascending or descending method; use of different intensity for the stimuli; assessment of uni- vs multi-modal TDT; etc) and our cohort was relatively older that those reported in the previous studies and therefore these factors might account for our results.

As far as the study of paired pulse suppression is concerned, we found reduced suppression of the N20 at ISI of 5ms (that is equivalent to the P27 of Tamura et al., 2010, since we measured the same peak-to-peak N20-P27 SSEP component) in patients when compared with the control group. However, we also found reduced suppression at ISI of 20 and 40ms. Frasson et al. (2001) as well reported reduced suppression at ISI of 20 and 40 ms, but not at shorter ISIs, in

patients with segmental and generalized dystonia (Frasson et al., 2001). Differently from Tamura et al. (2010) we elicited SSEPs by stimulation of the digital nerves of the index finger an not the median nerve at the wrist and the different finding might be related to the fact that SSEPs obtained from digital stimulation are more sensitive to changes in cortical inhibition.

A great body of literature supports the notion that SSEP suppression of the N20 at short intervals (ISI of 5ms) is mainly related to local inhibition at cortical levels (Eccles 1966; Meyer-Hardting et al., 1983; Emori et al., 1991; Soono et al., 1997; Araki et al., 1997), while suppression at longer ISIs (i.e. ISI of 20 and 40 ms) is more likely due to inhibitory post-synaptic interneurons within the dorsal column nuclei and the thalamus (ventral postero-lateral nucleus) (Eccles, 1966; Lueders et al., 1983). In our cohort, we found also reduced suppression of the SSEP P14 component, which is an additional proof of impaired inhibition at subcortical levels. Indeed, suppression of the P14 component of the SSEPs has been proved to reflect inhibitory activity within the dorsal column-lemniscus medialis (Lueders et al., 1983; Helmstaedter et al., 2009).

When studying lateral inhibition by means of double SSEPS, we also found reduced inhibition in dystonic patients versus healthy subjects. In the previous study by Tinazzi and coworkers, (2000), authors reported somehow similar results, but in this study the statistical significance was not reached. The significant difference that we found between the two groups might be due to the different procedures used in the two studies, as we tested lateral inhibition stimulating the thumb and index finger rather than two non-contiguous fingers as in the study by Tinazzi et al., (2010). Indeed, lateral inhibition is mediated by intra-cortical connections within a limited range (Helmstaedter et al., 2009) and given that contiguous fingers are represented adjacently in S1 (Kolasinski et al., 2016), it is likely that inhibition is stronger when

tested in adjacent fingers. Moreover, the difference in the sample size (19 vs 7 patients) might also explain the different result.

Our results of abnormal SSEPs findings in both temporal and spatial domains proved a pervasive deficit of sensory processing. However, the lack of correlation between spatial and temporal inhibition (i.e. SIR and STDT) suggested that increased STDT in dystonia is not just related to abnormal cortical activity and that further impairment within circuits processing the temporal aspects of afferent inputs are implicated in the abnormal temporal processing in dystonia.

When studying the HFOs, we found that patients had significantly reduced e-HFO area when compared to controls, while a similar non-significant trend was observed for l-HFOs. HFOs 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, while the late component represents activity of S1 inhibitory interneurons (Ozaki and Hashimoto, 2011). There was a single previous study testing HFOs in patients with cervical dystonia, reporting similar findings (Inoue et al., 2004). This further supports the notion that dystonic patients have decreased inhibition at different levels of the somatosensory system.

Correlations' analysis showed that only the suppression of the N20 at 5ms ISI and the l-HFO, i.e. measurements more likely related to local inhibition within S1 (Jones and Burton, 1976; Meyer-Hardting et al., 1983; Emori et al., 1991; Soono et al., 1997; Ozaki and Hashimoto 2011), correlated with STDT. Both N20 suppression and l-HFO are measures of temporal inhibition and, therefore, it might be inferred that inhibitory circuits within the S1 act sharpening the distinction between the first and the second afferent inputs in STDT (Conte et al., 2014).

The regression analysis additionally showed that a separate factor “dystonia group” was predictive of higher STDT. This suggests that there is one or more additional factors over and above our measures of cortical somatosensory inhibition that contribute to higher STDT in patients.

Previous neuroimaging studies in patients with abnormal STDT report contrasting findings, as structural and functional abnormalities have been found either at subcortical (putamen) (Giersch et al., 2009; Kimmich et al., 2014) and cortical (middle frontal, precentral and post central giri) levels (Frasson et al., 2001; Kimmich et al., 2014).

Of course, the nature of our study enables inferring whether the reduced inhibition in S1 is a primary/endophenotypic condition or a consequence of the pathology occurring at the level of the basal ganglia. Dystonia indeed should be construed as a network disorder. In line with this, we can conclude that higher STDT in dystonia can be largely, but not completely, explained by reduced cortical inhibition and the reported abnormal findings within the basal ganglia (Peller et al., 2006; Schneider et al., 2010) might play a supplementary role in modulating STDT.

As suggested (Conte et al., 2012; Conte et al., 2014), our results further advocate that inhibitory mechanisms within S1 might supposedly represent a therapeutic target to reverse abnormal STDT in dystonia. Of course it should be tested whether acting on the sensorimotor network with the aim to increase inhibition efficacy will in turn ameliorate overt manifestations/symptoms of dystonia.

Chapter 4

THIRD STUDY

Modulation of muscles' activity during sleep in patients with cervical dystonia.*

* **Antelmi E**, Ferri R, Provini F, et al. Modulation of the muscle activity during sleep in cervical dystonia. **Sleep** under rev

Background

Recently non-motor symptoms have been emerging as important determinants of the quality of life in patients with movement disoders, including dystonia (Stamelou et al., 2012). Various domains of quality of life, such as physical and social functioning and vitality, have been reported to be affected in patients with dystonia and among them, sleep has been consistently reported as a main complaint (Hertenstein et al., 2016). However, current treatment of dystonia deals mainly with motor symptoms, while, even if the relevance of non-motor has gained attention, so far their etiology and treatment options have been barely characterized. Moreover, sleep complaints in dystonic patients have been reported to be poorly responsive to botulinum toxin treatment and to deep brain stimulation (Stamelou et al., 2012). This underlines that sleep problems in dystonia deserve a discrete assessment, along with an ad-hoc treatment. In order to proprerly assess motor complaints in dystonic patients, therefore, it is important at first to understand their nature and pathophysiology and to compare subjective complaints with objective findings.

"A priori" thoughts lead to suppose the almost disappearance of dystonia during sleep. Indeed, according to the latest definition, dystonia is "*a movement disorder characterized by sustained or intermittent muscle contractions causing abnormal, often repetitive, movements, postures…..Dystonia is often initiated or worsened by voluntary action and associated with overflow muscle activation*" (Albanese et al., 2013). The relationship between dystonia and movement and posture leaps out as an instristic feature of dystonia itself and indeed, posture typically increases while holding a position or while performing movements, as observed with the phenomenon of the overflow of dystonia. This is even striking in task-specific dystonia, the appearance of which is tightly linked to certain movements (i.e. playing an instrument, writing, singing, and eating) (Quartarone et al., 2013). Also the other way around is true, with the

improvement of dystonia while resting, for example, while lying down or while holding the neck over a headrest (Lobbezoo et al., 1996; Jahanshahi, 2000).

On the other hand instead, physiologically, during sleep there should be a motor quiescence, with inhibition of the spinal motoneuron pool by supraspinal structures with consequent muscle hypotonia, until reaching an almost complete muscle atonia during REM sleep (Dement 1957; Chase 2005), but for brief phasic activity. Therefore, one would expect the disappearance of dystonia during sleep stages.

However, very few, spared and biased data have been produced on that regard (Hertenstein et al., 2015). Indeed, previous studies have been mainly conducted in small, non-homogeneous and non-controlled cohorts and there are no studies comparing objective findings (i.e. polysomnography - PSG) and subjective complaints. The spared data however seem to suggest disturbed nocturnal sleep as a non-motor feature of dystonia, frequently reported by the patients as affecting their quality of life (Soeder et al., 2009; Kuyper et al., 2011; Stamelou et al., 2012; Shukla et al., 2016). Prevalence of sleep complaints in focal dystonia has been reported to range between 40 and 70% (Hertenstein et al., 2015). However, so far subjective complaints have never been compared with objective evaluation of the architecture of sleep and with the presence of movement-related disorders or with the persistence of dystonia during sleep. Indeed, it is generally believed that all the movement disorders, but epileptic conditions, would disappear during sleep. However, the few polysomographic studies available so far reported instead the persistence, though “a minima”, of dystonia (Rondot et al., 1995; Sforza et al., 1991; Silvestri et al., 1990). The only exception is the study conducted at the Queen Square in 1996 by Lobbezoo and co-authors.

With this study we aimed therefore at investigating sleep complaints in patients with idiopathic isolated CD and to compare subjective complaints with objective evaluation of sleep

architecture by means of PSG, which is the gold standard to assess sleep and its related features (i.e. quality and sleep-related phenomena).

Materials and Methods

Population

We prospectively recruited twenty patients with idiopathic isolated CD, according to current criteria (Albanese et al., 2013), among those attending the outpatient clinics at the Institute of Neurological Sciences of the University of Bologna. In order to compare data, the tests have been also performed in twenty-two healthy subjects with similar age and gender distribution (and no reported family history for any neurological disorders, including dystonia). Additional exclusion criteria for both patients and healthy controls (HC) were: 1) no history of other neurological or psychiatric diseases and 2) no history of medications acting on the CNS. Patients were all on treatment with botulinum toxin injection and therefore investigations were conducted at the wearing-off of the treatment, i.e. at least 3 months after their last injections.

Procedure (**Figure 6**)

All the patients underwent a complete neurological examination including history taking, clinical examination, and brain MRI. In order to identify the main pattern of CD and the key muscles, we observed the patients in several different positions (i.e at rest, while sitting, while keeping the arms outstretched and while walking). Disease severity was assessed with the Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS). As regards sleep problems, both CD patients and HC underwent a thorough sleep interview excluding other sleep-related

problems, i.e. presence of obstructive apnea or snoring, presence of restless legs syndrome (RLS), according to current criteria (Allen et al., 2014). Subjective quality of sleep was evaluated by means of the Pittsburg Sleep Quality Index (PSQI) (Buysse et al., 1989), while the presence of excessive daytime sleepiness by using the Epworth Sleepiness Scale (ESS) (Johns 1991). Beck Depression inventory was used to investigate mood (Beck et al., 1961).

Both CD patients and HC underwent a full-night PSG, with the following standard montage: conventional EEG, bilateral electrooculogram (EOG), submentalis and anterior tibialis EMG, respiratory parameters, and electrocardiogram, infrared video (AASM, 2014). Additional EMG leads were put over the neck muscles affected with dystona: i.e. sternocleidomastoideus (SCM) and splenius bilaterally. Moreover, EMG leads were also put on the deltoid, contralateral to the most affected side, which served as control muscle. Sleep signals were sampled at 200 or 256 Hz and stored on hard disk in European data format (EDF) for further analysis.

Subjectively perceived discomfort/pain caused by dystonia was assessed at four different times, by means of a visual analogue scale (VAS, values 0 to 10) four times: 1) at the beginning of the recording (after all the technical procedures), 2) after 20 minutes of relaxed wakefulness with eyes closed (with subcontinuous alpha rhythm on the EEG) before sleep, 3) on awakening, the following morning, and 4) after 20 minutes of relaxed wakefulness with eyes closed (with subcontinuous alpha rhythm on the EEG) after awakening.

All experimental procedures were approved by the institutional ethic committee and conducted in accordance with the Declaration of Helsinki and according to international safety guidelines. Both CD patients and HC gave their signed informed consent.

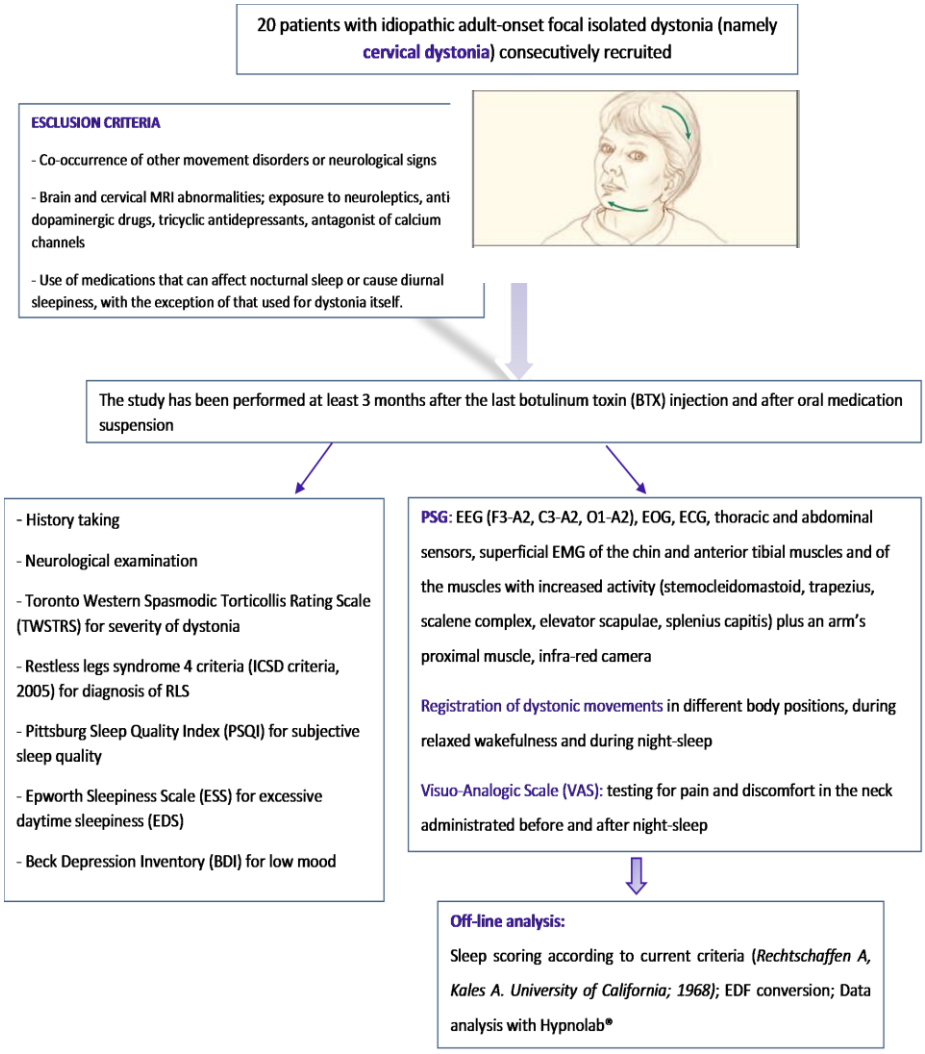
Sleep Staging and Muscle Activity Analysis

Sleep stages were scored according to the current criteria (AASM, 2014). After the scoring, we considered the following parameters: sleep latency (SL; defined as the first epoch of any sleep stage from lights off), and stage REM latency (REML) from sleep onset, total sleep time (TST), sleep period (SP; time from sleep onset to lights on), wakefulness after sleep onset (WASO), sleep efficiency (SE), absolute time spans and percentages (of total sleep time) spent in NREM sleep stages N1, N2, and N3, and in stage REM.

An ad-hoc software (Ferri et al., 2008) was implemented in order to analyze the EMG activity of muscles affected with dystonia and control muscles during the whole recording. The signal analysis was the result of the measurements and of the averaging-out of the amplitude of the rectified EMG signal during 1-second long mini-epochs and excluding all periods with large body movements or other types of technical artifacts.

Periodic leg movements during sleep (PLMS) were visually detected and marked and the PLMS index was calculated according to standard criteria (AASM, 2014).

Figure 6



Statistical analysis

The comparison between the CD patients and HC data was carried out by means of the non-parametric Mann-Whitney test for independent datasets, the Friedman ANOVA for multiple dependent datasets, or the chi-square test for frequencies, as appropriate.

Addittionally, a sequences of correlations were also performed by means of the multiple regression analysis and the calculation of the partial correlation coefficients. P value ≤ 0.05 was considered significant.

The commercially available software STATISTICA v. 8.0, StatSoft Inc. (2007) was used for all statistical tests.

Results

Demographic features, neurophysiological data and questionnaires results of CD patients and HC are reported in Table 3.

Table 3

Demographic features, neurophysiological parameters and questionnaire results obtained from patients and HC.

	CD (n=20)	**HC (n=22)**	**p≤ 0.005**
Demographic data			
Age, years ± SD	50.5±9.09	48.2±6.19	ns
Females/males, number	14/6	11/11	ns
Questionnaries (mean scores ± SD)			
ESS	3.8±2.5	2.4±2.8	ns
PSQI	6.8±5.6	2.3±2.1	**0.0009**
BDI	10.5±6.1	9.1±2.6	ns
Sleep Architecture			
Total Sleep Time, min ± SD	368.6±73.6	364.9±67.	ns

	6	8	
Sleep Efficiency, % ± SD	75.7±11.1	85±8.9	**0.005**
Sleep Latency, min ± SD	36.9±30.1	20.6±17.1	**0.038**
Sleep stage REM Latency, min ± SD	118.5±64.9	82.7±44.7	**0.042**
Sleep stage N1, % ± SD	10±5.5	8.6±6.3	ns
Sleep stage N2, % ± SD	48.2±12.5	46.7±8.5	ns
Sleep stage N3, % ± SD	20.6±8.8	22.4±9.2	ns
Sleep stage REM, % ± SD	20.4±5	18.2±3.9	ns
PLMS index, number/hour ± SD	4.3±6.2	5.5±10.5	ns
Arousal Index, number/hour	16.8±15.9	11.7±7.4	ns

ESS: Epworth sleepiness scale; PSQI: Pittsburgh sleep quality index; BDI: Beck depression inventory; PLMS: periodic limb movements during sleep; ns = not significant.

Patients had a mean disease duration (years ± standard deviation) of 7.6±5.7; mean value of the TWSTRS was 17.9±5.9. In 12 patients, dystonia was associated with tremor involving the neck. Patients and HC did not differ for age and gender distribution.

The results of questionnaires assessing excessive daytime sleepiness and mood complaints were comparable between the two groups, but CD patients had significantly increased pathological values on the PSQI, assessing subjective complaints of impaired nocturnal sleep. The results of the multiple regression analysis carried out with PSQI measuring subjective sleep quality as the dependent variable and age, disease duration, TWSTRS, ESS, and BDI as independent factors, in CD patients showed only BDI to be significantly correlated with PSQI (partial correlation 0.682, $p<0.0032$).

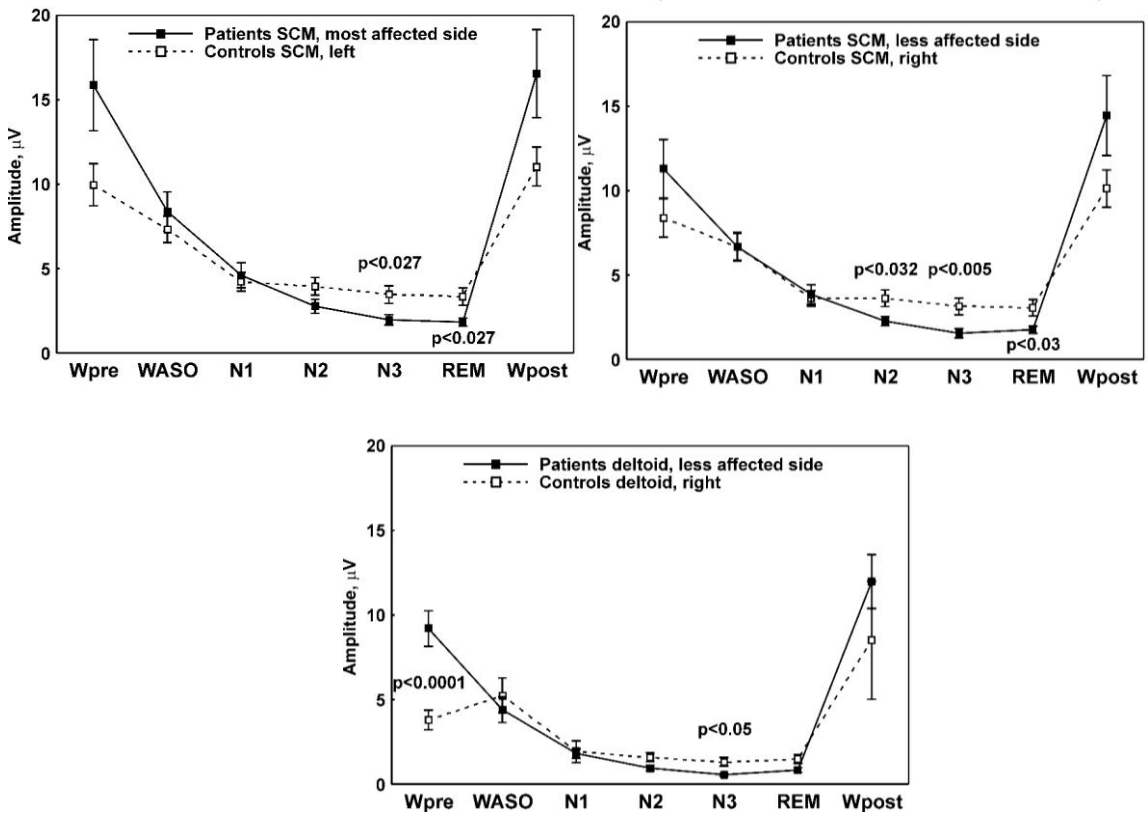
When analyzing sleep architecture, we found that CD patients and HC differed for SE, which was significantly reduced in the CD patients group ($p<0.005$); and SL and REML, which were significantly increased in the patients group ($p<0.0038$ and $p<0.042$, respectively). The multiple regression analysis performed considering the objective sleep parameters found to be abnormal in the above comparison, i.e. SE, SL and REML as dependent factors and all the clinical descriptors (age, disease duration, TWSTRS, ESS, PSQI, and BDI) as independent factors, in CD patients, disclosed only a significant negative correlation between SE and PSQI (partial correlation -0.533, $p<0.04$) and a significant positive correlation between SL and PSQI (partial correlation 0.524, $p<0.048$).

Analysis of Muscle Activity

Muscle activity during relaxed wakefulness (patients lying supine in bed) pre (Wpre) and post-night sleep (Wpost) over the muscles affected with dystonia (both the most affected one and the contralateral one) was slightly, but not significantly, higher than that of HC (Figure 2). In Wpre, activity over the control muscle (i.e. deltoid contralateral to the most affected side) was significantly increased in CD patients, when compared to HC. In the different sleep stages (i.e. stage N1-N2, N3 and REM), activity of dystonic muscles and control muscle progressively decreased from Wpre to N1, reaching significantly decreased values compared to HC in N3 and REM sleep stages in the most affected side, in N2, N3 and stage REM in the less affected side and in N3 in the control muscle (**Figure 7**).

Figure 7

Comparison between the amplitude of the EMG signal in the different pairs of corresponding muscles recorded in patients and controls in the different wakefulness periods and sleep stages considered in this study.

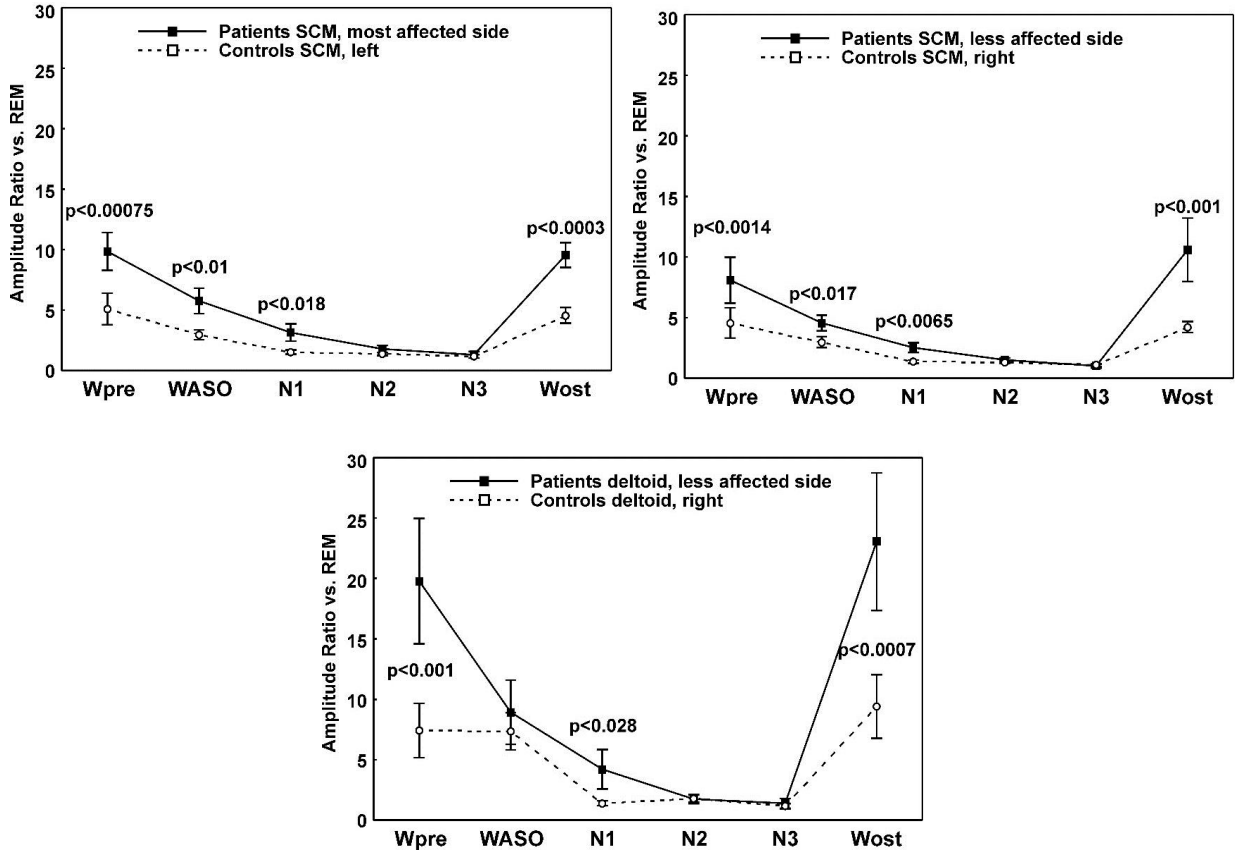


***Legend**: µV: microvolts; N1, N2, N3: stage 1, 2 and 3 of NREM sleep; REM: REM sleep; SCM: sternocleidomastoids; WASO: wakefulness after sleep onset; Wpre: wakefulness pre-night sleep; Wpost: wakefulness post-night sleep.*
Values are shown as mean (squares) and standard error (whiskers).

When the muscle activity amplitude values were normalized by subtracting the individual mean amplitude during sleep stage REM (which is considered to be the gold standard for muscle atonia/hypotonia),[18] even if patients had a significant relative higher muscle activity over the neck muscles in Wpre, Wpost, WASO and N1, they finally reached the same levels of muscle activity of HC in N2, N3 and stage REM (**Figure 8**).

Figure 8

Comparison between the individually normalized amplitude (difference from that of sleep stage REM) of the EMG signal in the different pairs of corresponding muscles recorded in patients and controls in the different wakefulness periods and sleep stages considered in this study.



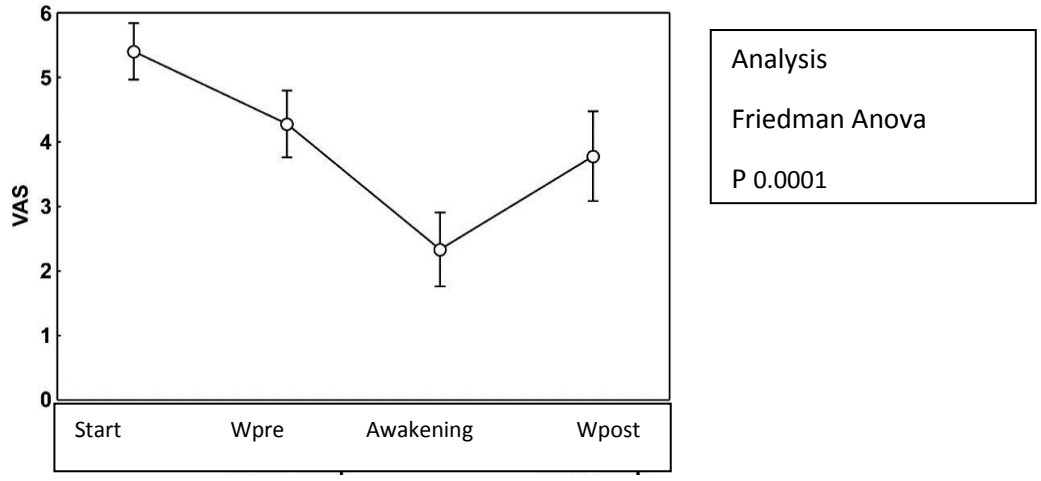
***Legend**: µV: microvolts; N1, N2, N3: stage 1, 2 and 3 of NREM sleep; REM: REM sleep; SCM: sternocleidomastoids; WASO: wakefulness after sleep onset; Wpre: wakefulness pre-night sleep; Wpost: wakefulness post-night sleep.*
Values are shown as mean (squares) and standard error (whiskers).

Differently from the objective measurement of the muscle activity that soon after sleep returned to the values recorded before sleep, patients reported the severity of neck discomfort/pain to be reduced after nocturnal sleep as evident from the Friedman ANOVA

(p<0.00001) carried out on the four VAS evaluations obtained before and after night sleep (**Figure 9**).

Figure 9

Subjective level of discomfort/pain caused by dystonia in patients evaluated by means of a visual analogue scale at four different times, before and after sleep.



Legend: *VAS: visual analogue scale for pain/discomfort. Wpre: wakefulness before sleep onset; Wpost: wakefulness after nocturnal sleep.*
Values are shown as mean (squares) and standard error (whiskers).

There was no statistical correlation between the different VAS evaluations and the objective muscle activity measurements during relaxed wakefulness before and after sleep.

Discussion

The study at issue is the first one systematically analyzing the activity on dystonic muscles during the different sleep stages and comparing neurophysiological data with the results of self-reported questionnaires assessing excessive daytime sleepiness and quality of sleep in a

representative cohort of patients with idiopathic isolated CD, compared to age- and sex-matched HC.

Results of questionnaires aimed at investigating subjective complaints showed a significantly reduced quality of sleep assessed by means of the PSQI in CD patients, when compared to HC. This finding is in line with results of previous studies (Trotti et al., 2009; Avanzino et al., 2010; Paus et al., 2011). The analysis of the correlation between clinical descriptors and subjective complaints of nocturnal sleep disclosed only a weak correlation with higher scores on the BDI, confirming previous findings, which reported on the mutual link between quality of sleep and mood in dystonia (Avanzino et al., 2010; Paus et al., 2011; Eichenseer et al., 2014) and generally confirming the impact of mood deflection in quality of sleep (Palagini et al., 2013). As reported also in previous cohorts (Trotti et al., 2009; Avanzino et al., 2010; Paus et al., 2011; Eichenseer et al., 2014), severity of dystonia and disease duration did not seem to affect the subjective perception of sleep quality. Even if patients had a significantly affected quality of sleep, they did not complain of excessive daytime sleepiness, assessed by means of ESS, where values were in the range of normality and comparable with those reported in the control group. Previous studies in cohorts with different types of focal dystonia reported as well the absence of EDS (Avanzino et al., 2010; Paus et al., 2011; Eichenseer et al., 2014), while there is a single study (Trotti et al., 2009), which finds instead higher values of EDS at the ESS in dystonic patients respect to HC.

As far as sleep architecture analysis is concerned, our study showed a significantly decreased SE with difficulty in falling asleep (increased SL) and in reaching REM sleep (increased REML). Arousal index tended to be higher in CD patients as well, but this value did not reach statistical significance. Previous PSG studies reported similar findings. Sforza et al. (1990) in a cohort of 10 patients with cranial dystonia found decreased SE, slow-wave sleep (SWS) and REM sleep,

while increased SL and arousals. Decreased SE and increased REML were also reported in patients with CD (Lobbezoo et al., 1996; Randot et al., 1995). In our cohort, however, parameters measuring sleep architecture did not correlate with clinical descriptors, and particularly with disease severity or disease duration. On the contrary, instead, higher scores at the PSQI, which measures the level of subjective complaints of sleep impairment correlated with decreased SE and increased SL at the PSG recordings, showing therefore an overlap between subjective complaints and objective measurements of impaired sleep architecture.

As concerns additional sleep complaints or complaints of other sleep diseases of sleep-related movements, there is a single study reporting on an increased incidence of RLS and bruxism in dystonic patients when compared to controls (Trotti et al., 2009). Our study, instead, cannot confirm these data as none of our patients reported symptoms suggestive of additional sleep-related disorders and PSG as well failed to document any of these conditions, being the PLMs index also comparable with that of the HC.

The analysis of EMG muscle activity during the PSG recording showed that activity over the muscles affected with dystonia, and even over the control muscle, progressively decreases and nearly disappears in SWS and REM sleep. Indeed, even if patients had (although not significantly) increased amplitude of muscle contraction during relaxed wakefulness, this amplitude reaches values which are significantly lower than those of HC, during both SWS and REM sleep. These data are somewhat different with what has been previously reported. To be honest, there are only few studies objectively evaluating muscle activity during sleep. Three of them reported persistence of muscle activity over the dystonic muscles both in patients with cranial dystonia (Sforza et al., 1991; Silvestri et al., 1990) and with CD (Rondot et al., 1995). In the study by Sforza et al. (1991) the number of spasms per hour of sleep in the cranial region (patients with blepharospasm or Meige syndrome) progressively decreased but did not

disappear during the night and then gradually increased, particularly prior to awakening. Spasms were rarely associated with an EEG arousal. Silvestri et al. (1991) reported fairly similar results, in a cohort of seven patients with cranial dystonia. However, previous studies have been conducted in small and non-controlled cohorts (Rondot et al., 1995; Sforza et al., 1991; Silvestri et al., 1990) and data are reported in a descriptive fashion, without performing any statistics (Rondot et al., 1995). There is only one controlled study, comparing findings in nine patients with CD versus nice HC (Lobbezoo et al., 1996) and describing an improvement of dystonia linked to the supine position and the virtual disappearance of muscle activity with sleep. Indeed, abnormal cervical muscle activity was reported to decrease immediately when lying down, without the intention to go to sleep and then to be gradually abolished in all patients during the transition from relaxed wakefulness to light NREM sleep. Finally, during sleep any muscle activity could be detected on muscle affected with dystonia (Rondot et al., 1996).

Our study, along with confirming the virtual disappearance of dystonic activity during SWS and REM sleep, showed also a significant decrease in muscle activity over the dystonic muscles and over the control muscle, when compared to muscle activity recorded on homologous muscles in HC. This is of interest and it can be hypothesized that muscle affected with dystonia obey to the sleep-promoted homeostatic balance (Vyazovskiy et al., 2015). Indeed, it can be that dystonia muscles, which had been over-active throughout the day need a special and deeper rest at nighttime. In animal models, indeed, it has been reported that during nighttime, hypotonia in cervical muscles is greater if compared with cranial muscles (Lu et al., 2005; Kato et al., 2007).

Unfortunately, studies reporting on modulation of EMG muscle activity at nighttime after exercise or training are lacking. However, indirect observations might suggest that increased

activity during daytime induces homeostatic recovery during nighttime (Kleitman, 1963; De Mello et al., 2002). Pioneering studies reported a major decrease in the EMG activity of antigravity skeletal muscle tone during sleep (Kleitman, 1963), except for leg muscles (Okura et al., 2006), suggesting somewhat a recovery function of sleep for those muscles more active during daytime.

The role of sleep in synaptic plasticity has been extensively reported, as sleep seems to weak synapses through a process called ‘‘synaptic renormalization’’ (Tononi and Cirelli, 2012). The mechanisms governing renormalization are not completely known. Synaptic scaling (or homeostasis) is characterized by a global adjustment of synaptic weights in a neuron or network which is proportional to the strength at each synapse. Synaptic downscaling occurring during sleep therefore would offset Hebbian long-term potentiation, which if left unconstrained would result in continuous synaptic strengthening that would saturate a neuron or neuronal network’s ability to form new synapses (Turrigiano et al. 2008). Therefore, synaptic scaling should promote adjustment of synaptic weights that allows the network to avoid network instability (i.e., a saturation of synaptic strength). In respect to these arguments, sleep in dystonia might at least partly try to rescue the system and to prevent ‘‘synaptic overload’’. Of course, the nature of our study prevents any conclusions on this topic and future studies with a more extensive evaluation of muscle activity and of neurophysiological correlates are warranted in light also of the potential related therapeutic advantages.

Another explanation might be that during sleep impaired connectivity and plasticity instristic to dystonia is somehow overcome. Indeed dystonia is known to be a network disorder with impaired inhibition being the main neurophysiological drive (Quartarone and Hallet, 2013; Antelmi et al., 2016). During sleep instead there is an interruption of cortical effective connectivity (Massimini et al., 2015) and, during REM sleep, basal ganglia seem to be bypassed

by the pyramidal system (De Cock et al., 2011; Mayer et al., 2015). It is possible therefore that sleep may somehow reset abnormal connectivity and inhibition in dystonia, and that during sleep the sensorimotor cortex, released from the basal ganglia influence, might function in another way, "*getting rid of dystonia*". We cannot exclude also a contribution of the GABAergic inhibitory system as from one side, hypotonia during sleep is thought to be induced by glycinergic and GABAergic post-synaptic inhibition (Chase, 2013) and from the other side, abnormal the GABAergic inhibitory system within the CNS seem also to play a role in dystonia pathophysiology (Antelmi et al., 2016; Garibotto et al., 2011).

Orexin system, instead, which is known to promote wakefulness and motor activity during daytime, and rest/hypotonia and sleep during nighttime (Hu et al., 2015) and to orchestrate central motor control in a homeostatic regulation manner, has never been investigated in patients with dystonia.

Additionally, the finding of a statistical significant sleep benefit on cervical pain/discomfort, evaluated by means of VAS soon after awakenings, further supports the homeostatic function of sleep for dystonic muscles.

During the relaxed wakefulness preceding sleep, while CD patient are lying down with head and neck in a rest position, activity on a proximal muscle contralateral to the most affected side was found to be higher than that of the homologous one in HC. This phenomenon might be interpreted as a form of overflow, related to the abnormal sensorimotor integration in dystonia (Quartarone and Hallet, 2013).

To conclude, or study showed a significantly affected quality of sleep in dystonic patients, when compared to HC. Subjective complaints are confirmed by objective measurements of sleep, which showed an affected sleep architecture with reduced efficiency of sleep and increased of latency prior to falling asleep. Impaired nocturnal sleep did not correlate with

measurements of disease severity neither with the persistence of muscle activity over dystonic muscles, which was instead found to disappear during nighttime. Therefore, other factors, over and above, must be related to the subjective perception of poor sleep quality and to the abnormal sleep architecture. These findings are in line with what has been reported in previous studies, and in fact botulinum toxin injections have been reported to significantly improve dystonic symptoms, without having benefit on sleep (Eichenseer et al., 2014). Yet, as in our cohort, also previous studies could not find a correlation between sleep impairment and severity of dystonia (Avanzino et al., 2010; Paus et al., 2011).

It remains to capture the mechanisms driving to the abnormalities affecting sleep macrostructure in patients with dystonia. This is pivotal in order to choose the appropriate treatment and in order to meet patients' needs and to improve their quality of life. Moreover, developing studies aiming at discovering the specific pathophysiological substrates will be instrumental in understanding the pathophysiology of dystonia itself and the true health impact of sleep disorders on dystonia. In that regard, in the future additional computerized analyses aiming at characterizing the microstructure of sleep might shed light on this mechanism.

It remains also to be tested whether drugs known to improve self-reported sleep quality (Baandrup et al., 2016) or targeting sleep efficiency (like instaminergic or orexinergic antagonists) (Herring, 2012) might be efficacious also in dystonic patients.

Chapter 5

Conclusions and general remarks

Dystonia is a syndrome, with clinical and etiological heterogeneity, but with a common final pathway leading to “abnormal postures”. Although a great effort has been made, the complete understanding of its pathophysiology is still pending and this is probably related to the paucity of animal models for translational studies, the absence of a consistent pathological substrate and the highly variable phenotypes and genotypes.

Overall, the genetic make-up along with epigenetic and environmental factors all play a role in its “epiphany”.

Epidemiological studies suggested a correlation with environmental triggers (like trauma) or repetitive highly-skilled manual performance. Of course, environmental triggers and modifiers should act on a fertile ground, i.e. on a genetic predisposition. Among all the abnormalities that have been reported, abnormal plasticity arises as the “main” consistent one, likely related to the lack of inhibition (Hallet, 2011).

Aberrant neuronal plasticity of a motor learned program by repeated practice holds important clue to the etiopathology of dystonia. Indeed, external triggers acting on a predisposed brain might induce synaptic changes generating aberrant motor engrams. The pathophysiology of this has been proved to be related to the lack of inhibition. Basal ganglia filter and modulate inputs to improve the precision of fine movements. The failure of surrounding suppression has been related to deficient inhibition by basal ganglia gabaergic interneurons and output (Hallet, 2011), but it is likely that other structures, as shown also in our (Chapter 3) and in previous study (Tamura et al., 2008; Antelmi et al., 2017) might have additional role. The failure of inhibition clearly fits with the

clinical picture of dystonia. Indeed, one of the main characteristics of this disorder is its tight link with movement.

Oppeheim (1911) said in its original description: *"muscle tone was hypotonic at one occasion and in tonic muscle spasm at another, usually, but not exclusively, elicited upon voluntary movements*", and similarly the last consensus on dystonia acknowledged that "*Dystonia is often initiated or worsened by voluntary action and associated with overflow muscle activation."* Yet, another important feature is the predictability and patterned nature of the muscle, showing that movements might trigger aberrant motor engrams.

The system is imbalanced also at the level of the sensory system. One emerging theory is that sensorimotor systems have hebbian-like plasticity (Weise et al., 2009). In a dystonic endophenotype, the summation of abnormal sensorimotor plasticity and the inability to control homeostatic mechanisms may therefore result in a chaotic reorganization of sensorimotor maps. In this regard, STDT has been proved to be endophenotypic in dystonia. Our study (Chapter 3; Antelmi et al., 2017) further confirms this finding, by reporting abnormal values in both affected and non-affected sides, and without demonstrating a correlation with the severity of symptoms. Moreover, our results go further showing a link of abnormal sensory perception and measurements of inhibitions. Both cortical (local) inhibition within the sensory cortex and thalamo-cortical mediated inhibition are abnormal in our cohort of patients with CD, indicating a spread impairment of processes of inhibition. However, abnormal STDT correlated only with measurements related to local inhibition. Our model could partially explain abnormal STDT values, as the regression model showed that only 64% of abnormal temporal processing could be explained by these findings. This means that other factors, over and above, play an additional role in keeping with the idea that dystonia should be construed as a network disorder.

In keeping with this concept, lately it has been pointed out that along with the basal ganglia and the sensorimotor cortices, also the cerebellum might play an additional role in this disorder (Prudente et al., 2014). The cerebellum has been held responsible for contributing to the deficit of sensorimotor integration presented in dystonia, as it might process afferent proprioceptive information and modify the threshold of the somatosensory cortex through the cerebello-thalamo-cortical loop (Filip et al., 2017). It could also influence the cortex plasticity (Lehéricy et al., 2013). The results of our study (Chapter 2), testing cerebellar function by means of EBCC paradigm, showed that this is abnormal in dystonia when compared to controls, but it also shows that abnormal cerebellar functioning segregates with the presence of tremor, rather than with dystonia itself. Therefore, it is possible that along with the main manifestation of dystonia, peculiar aspects could instead be brought by different degrees of involvement of a node of the networks, explaining therefore the phenotypic variability of dystonia.

Finally, lately non-motor symptoms are emerging as important determinants of the quality of life in patients with movement disorder. Virtually, all the movement disorders disappear or improve while sleeping. Exceptions are of course sleep-related movement disorders. Nocturnal occurrence of movements, indeed, leads firstly to the suspicion of a parasomnia or of a seizure. The only exceptions seem to be dystonia due to ADCY5 mutation (Chen et al., 2015) or the wide spectrum of phenotypes mixing dyskinesia, ataxia and paroxysmal hypnogenic diskynesia due tu PRRT2 mutation (Liu et al., 2016). However, in the past there have been some suggestions that dystonia might persist during sleep (Sforza et al., 1990; Silvestri et al., 1991). Moreover, subjective complaints of patients about sleep have been consistently reported (Eichenseer et al., 2014), but never investigated by means of objective evaluation. Our study (Chapter 4) showed important clues on that regard. Interestingly, indeed we found subjective complaints to correlate with abnormal sleep architecture as measured with overnight PSG. However, dystonia clearly

disappears during sleep, until reaching values of muscle activity relatively lower when compared to the activity recorded from the homologous muscles in heathy controls. This leads to speculate on a possible homeostatic role of sleep for dystonia, as proposed for the sleep benefit in parkinsonian conditions. Of course, the nature of our study hampers any conclusion on that matter.

Overall, what we clearly demonstrated was the presence of an impairment of objective measurements of sleep quality. This seemed to be independent from dystonia severity and from disease duration, implying that sleep abnormalities might be ascribed independently to dystonia network and that they deserve a discrete look and an appropriate therapeutic approach.

To conclude, our results further show that dystonia is not merely a movement disorder and that abnormalities of physiology go behind the basal ganglia, and encompasses several nodes of the central nervous system, such as the cerebellum (Chapter 2) and the sensorimotor cortex (Chapter 3). Overall, dystonia seems to arise from different types of defects within the sensorimotor network and dysfunction may involve a single node within the network, or possibily even result from the simultaneous dysfunction of more than one node, or abnormal communication between the nodes. Within this network, involvement of a node more than another may exaplain phenotypic variability. Yet, network embalance and aberrant inhibition might account also for the emergence of non-motor features (Chapter 4), while the sleep-related improvement might theoretically be ascribed to the different connectivity linked to the state of being of sleep (Massimini et al., 2015).

Improved understanding of pathogenetic pathways will hopefully lead to the discovery of new therapeutic targets aiming to provide better symptomatic and, optimistically, etiological treatment in dystonia.

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