Cervical dystonia

Abnormal cerebral activation patterns related to preparation and execution of hand movement

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Contents

Chapter 1

General introduction

Introduction

The theme of this thesis concerns the cerebral organization of movement in patients with cervical dystonia (CD). The focus lies on the essential role of the sensory system to integrate sensory information for the preparation of movement.

CD, a type of focal dystonia, is a movement disorder that occurs when a patient intends to perform a movement. The distinct dystonic symptoms such as excessive muscle contraction and overflow to adjacent muscles are seen as an impaired inhibitory effect of the basal ganglia on the cortex. However, patients also report sensory abnormalities that can not easily be linked to basal ganglia impairment. Sensory tricks and the development of focal dystonia after sensory trauma are widely recognized (Jankovic, 2001; Schramm et al., 2004). Abnormal sensory input can affect movement performance when the integration of sensory information for the preparation of movement is abnormal (Abbruzzese and Berardelli, 2003). Indeed, several studies have shown that movement of the affected body part is related to abnormal sensory processing and sensorimotor integration (Ceballos-Baumann et al., 1995; Odergren et al., 1996; Odergren et al., 1998; Pujol et al., 2000). Structural abnormalities have also been reported in the sensorimotor regions in focal dystonia (Delmaire et al., 2007; Draganski et al., 2003; Egger et al., 2007). In addition, recent studies point out that movement and sensory stimulation of non-affected body parts also demonstrate abnormal sensory input (Siggelkow et al., 2002), abnormal information processing in the sensorimotor regions (Feiwell et al., 1999) and abnormal movement performance which may not be evident at a clinical level (e.g. subclinical abnormalities) (Carboncini et al., 2004).

Movement execution requires sensory input from particularly the proprioceptive and visual systems. The visual system estimates the distance between the limb and an object, while the proprioceptive system provides sensory feedback of the performed movement (Felician et al., 2004). However, it is possible to imagine a movement without actually performing it, and without sensory feedback (Stephan et al., 1995). This points at a feedforward mechanism of the sensory system that predicts sensory consequences of the upcoming action (Blakemore et al., 1998).

Thus, the sensory system is not only involved in movement execution but already in movement preparation. With Positron Emission Tomography (PET) or functional Magnetic Resonance Imaging (fMRI), cerebral activations during movement performance can be measured. Such a movement task activates movement preparation and execution networks, but is also blurred by sensory feedback mechanisms (Stephan et al., 1995). Movement imagery has been introduced as a task that reflects movement preparation without subsequent execution (Jeannerod and Decety, 1995). As sensorimotor integration is part of movement preparation, movement imagery may thus, at least partly, reflect cerebral activation during sensorimotor integration.

Abnormal cerebral activation has been described for movement execution in a dystonic body part (Playford et al., 1998; Preibisch et al., 2001). A recent study also underscores abnormal sensory processing in non-dystonic body parts (Feiwell et al., 1999). It is not clear, however, if these abnormalities can be linked to abnormal sensorimotor integration. Since CD has normal movement in all body parts except for head and neck (Dauer et al., 1998), a simple hand movement task would allow for investigation of abnormal cerebral activation during clinically normal movements. Movement performance of non-dystonic body parts may, however, already be impaired at subclinical level (Carboncini et al., 2004). In this respect, electromyography (EMG) is useful to demonstrate a relation between abnormal activation patterns and subclinical abnormal muscle activity.

To understand patterns of cerebral activation related to sensorimotor integration and clarify any abnormal activation patterns in CD, it is necessary to look at sensorimotor integration in healthy controls (HC) during similar types of movement execution and imagery. In addition, “inducing” impaired sensorimotor integration in HC may reveal changes in network activation, possibly similar to CD. Changes may include regional decreases of activation, reflecting local deterioration of function while increases in other regions might point at attempts to compensate such deterioration. Transcranial Magnetic Stimulation (TMS) is a non-invasive technique that can induce a temporary functional lesion of a target area. TMS interleaved with fMRI is able to directly obtain the TMS-induced effects by measuring changes in activation patterns. Also, application of this technique to an

already impaired sensorimotor network in CD may show to what extent remote cortical networks are able to adapt to impaired cortical function. This impaired function in CD may correlate with structural abnormalities as seen in other focal dystonias using diffusion tensor imaging (DTI)(Delmaire et al., 2007; Draganski et al., 2003).

Cerebral organisation of movement execution and imagery

Planning, preparation and performance of movement is organized by a complex cortical network. Several levels of processing can be distinguished. The main output channel for execution of movement is the primary motor cortex (M1) from where the pyramidal tract originates and projects to the spinal cord. Planning and preparation of movement is performed in higher-order motor areas (Georgopoulos, 1991; Rizzolatti and Luppino, 2001), containing parallel networks in the prefrontal, premotor and parietal cortices. The prefrontal region has a particular role in movement selection and recruitment of memorized concepts of movement (Tanji and Hoshi, 2001). The parietal region controls the higher-order sensorimotor integration of the desired movement (Leiguarda and Marsden, 2000; Mountcastle et al., 1975). The posterior areas process predominantly visual information, whereas the anterior areas are related to somatosensory information and integration of somatosensory and visual information (Caminiti et al., 1996; Rizzolatti et al., 1997). The premotor region is the main connector between higher-order motor areas and M1. The dorsal part directs movements to M1 based on a combination of somatosensory and visual information from the parietal lobe while the ventral part contributes to the control of hand movement required for the manipulation of objects and functions on a more cognitive base relating to the understanding of movements (Chouinard and Paus, 2006). Supporting systems as basal ganglia and cerebellum are important for the refinement and evaluation of movement. The basal ganglia are particularly concerned with the selection of adequate responses to a particular situation and the suppression of inadequate responses. In the basal ganglia, the putamen “receives” information from all parts of the cerebral cortex. This information projects through an indirect pathway, containing globus pallidus externa and subthalamic nucleus, or direct pathway to

the globus pallidus interna. The striatal output of the basal ganglia has an ascending component to the thalamic nuclei that in turn are connected with the premotor and prefrontal cortical areas, and a descending component to the mesencephalon that projects to the lower brain stem and spinal cord (Alexander, 1994; Alexander and Crutcher, 1990). The cerebellum is associated with balance and monitoring of the outcome of movements by sensory feedback which is in turn used to learn movement (Jueptner et al., 1997; Morton and Bastian, 2007). Nearly all cortical areas project to the cerebellum. Separate output channels of the cerebellum concerned with different aspects of movement project via the thalamus back to generally the same cortical regions from where they originated (Kelly and Strick, 2003).

Sensorimotor integration

Sensory information is crucial for the preparation of movement. Movement cannot be performed without knowing where one's own body parts are in relation to the rest of the body (i.e. a functioning body scheme) and external space. In the past, potential abnormalities in the sensory system were hardly taken into account in movement disorders studies. It was assumed that dystonic symptoms, especially the excessive muscle contraction and overflow to adjacent muscles, were related to the tendency of the motor system to become hyperactive due to impaired inhibitory effects of the basal ganglia on the cortex. In recent years, this hypothesis was recognized to be too simplistic, since it does not take into account the sensory symptoms. Both the motor system and the sensory system are involved in dystonia (Abbruzzese and Berardelli, 2003). This consideration led to a new hypothesis that dystonia does not occur due to abnormalities in movement execution but in movement preparation. It is still debatable whether impaired movement preparation is brought about by impairments in sensorimotor integration or in processing of sensory information.

The discrimination of somatosensory input from different parts of the body (for example between little finger and thumb) relies strongly on information processing in the primary somatosensory region (S1), while integration in the secondary somatosensory region (S2) enables its contribution to movement preparation or

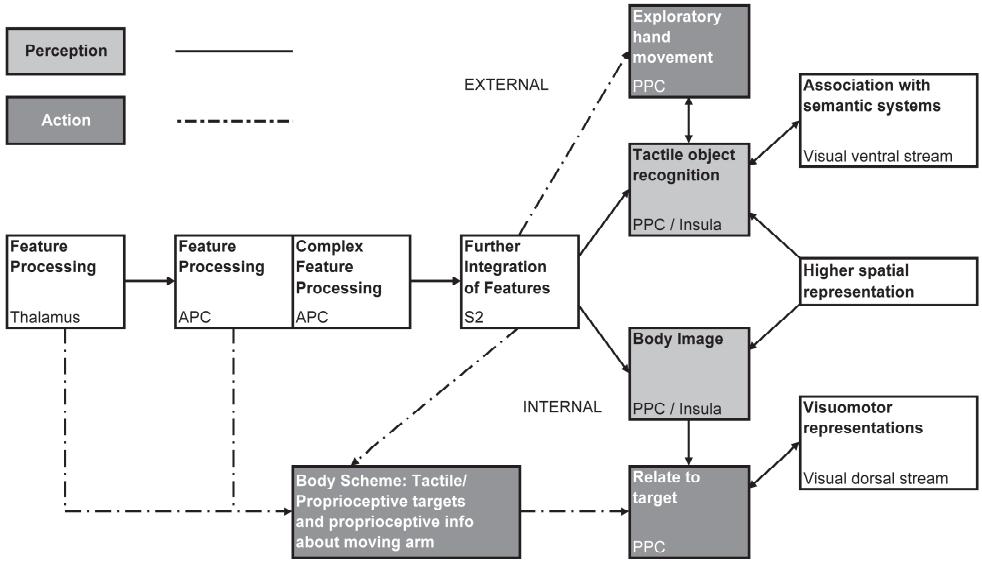

Figure 1 – *Schematic model of somatosensory information processing. This model shows distinctions between action and perception, and between processing of somatosensory information for internal stimuli (e.g. where you have been touched) and external stimuli (e.g. shape of objects). It also demonstrates the progressive integration of different stimulus features. Early processing is mainly concerned with simple features (e.g. stimulus location and duration), subsequent processing involves detection of the direction and velocity of a target moving over the body surface. Higher association areas combine these features with other information streams to provide information about the shape of an object or integrate it in a representation of the body. APC = anterior parietal cortex; S2 = secondary somatosensory cortex; and PPC = posterior parietal cortex. Adapted from Dijkerman & de Haan 2007. Somatosensory processes subserving perception and action. Behavioral and brain sciences 30;189-239. Copyright Cambridge University Press 2007.*

other cortical actions (Figure 1) (Hinkley et al., 2007). Two types of sensory information are in particular important for movement preparation. One type helps to maintain an internal representation of the body and the other of the external world, which is essential to estimate for example the distance between limb and an object or the position of that specific limb in comparison to the body (Felician et al., 2004). Visual information, related to representations of external space, is required when one wants to reach for an object. Information about the object location is linked to movement direction, while object shape is linked to positioning of the fingers (Tunik et al., 2005). This information about external space has a particular right-sided

lateralization in the posterior superior parietal cortex along the intraparietal sulcus extending to the occipital lobe (de Jong et al., 2001; Naito et al., 2005). Proprioceptive information, related to representations of body scheme, has a more left-sided lateralization in the anterior intraparietal sulcus extending to the inferior parietal lobe (de Jong et al., 2001). Proprioception from the moving limbs provides the brain with a sensory feedback of the performed movement. To distinguish this sensory information about oneself from external sensory information, the higher-order motor regions generate corollary discharges during movement (McCloskey, 1981). These signals are not part of the generation of movement, but interact with the processing of sensory information of the self (Poulet and Hedwig, 2007). The corollary discharges are proposed to be a part of an internal feedforward model, created to predict the sensory consequences of the upcoming action (Blakemore et al., 1998). Movement may thus be associated with the actual movement plan and a copy of this plan that provides information about the expected sensory consequences of the movement, also called efference copy (Cullen, 2004; von Holst and Mittelstaedt, 1950). Sensory feedback remains important to correct the feedforward model. Interestingly, movement can also occur without proprioceptive feedback, on the basis of efference copies only. Modulation of the sensory information is then mediated by increased interaction of the S1 with the premotor cortex, supplementary motor area (SMA), intraparietal cortex and cerebellum (Christensen et al., 2007).

Movement imagery

Movement (or motor) imagery is characterized by vivid mental representations of a given movement performance which is internally rehearsed in working memory without any overt movement output (Decety, 1996). Movement imagery may be experienced in two ways: the first-person perspective shows internal or kinaesthetic images, corresponding to the kinaesthetic representation of the action from within. The third-person perspective is an external or visual image involving a visuospatial representation of the action. In other words, in the first person perspective, the subjects feel themselves executing movements while in the third

person perspective, the subjects see someone else executing movements (Jeannerod, 1995).

Movement imagery is a neuronal process which involves specific brain structures that also participate in the execution of actual movements. In addition, results from neuroimaging studies show that movement imagery is in the same class of neural processing as movement preparation and planning (Ehrsson et al., 2003; Porro et al., 1996). Efference copies are thought to supply sensory information for movement preparation. Since movement imagery seems to reflect movement preparation, efference copies are required for movement imagery as well. These copies are not only used to anticipate and cancel out the sensory consequences of a given movement, but also stabilize motor systems in the presence of sensorimotor feedback delays or absence of sensory feedback (e.g. in movement imagery) (Cullen, 2004). For this thesis, comparing movement imagery with movement execution has especially been useful to assess integration of sensory information, since imagery does not entail real movement related sensory feedback (Stephan et al., 1995). We assume that movement imagery by itself uses efference copies as the only “sensory information”. Although movement imagery recruits the same preparation networks as movement execution, some regions are more or less involved during imagery compared to execution of movement. This particularly holds for M1. While fMRI studies have shown activation in the contralateral M1 in movement execution and sometimes in imagery, PET studies do not show this M1 involvement during imagery. This controversy may result from the use of different behavioural tasks. When imagery is performed as a preparation of movement, it is likely to include motor executive regions like the M1. When the movement imagery task is a mental operation of sensorimotor representations, for example mental rotation of body parts, M1 will not be recruited as part of the brain activation network. Also, if subjects are not trained to use kinaesthetic imagery, they may visualize the movement instead of feeling the imagery come from within. In that case the imagery movement is likely to involve the occipital cortex and not M1 (Hanakawa et al., 2003; Lotze et al., 1999). In addition, the left superior parietal region may be more activated during imagery than execution of movement, since the “storage” of mental images is assumed to be localized here. Patients with left

superior parietal cortex lesions were not able to imagine both hands, while patients with right parietal cortex lesions only showed imagery deficits of the contralateral hand (Sirigu et al., 1996).

Focal dystonia

Dystonia was first described by Kopp (1836) who reported cramps and occupational spasms during writing in a fifty-year old man. Later, Hammond (1871) and Barraquer-Roviralta (1897) mentioned forms of generalized dystonia under the term athetosis. It was the German physician Oppenheim who first used the term ***dystonia***. He described four cases with dystonia musculorum deformans, a rare hereditary neurological disorder causing generalized spasms, contortion of muscles and abnormal tone with coexistent hyper- and hypotonia (Oppenheim, 1911). During the next 30 years, physicians argued whether dystonia was indeed a neurological disorder or that coexisting symptoms such as hysteria and disfiguring postures were more pronounced so that dystonia may be a psychiatric disorder. In 1944, Herz brought dystonia back to the domain of neurology by analyzing pictures frame-by-frame of dystonic movements. He concluded that these movements were slow, long-sustained, powerful and non-patterned contortions of axial and appendicular muscles that could spread from one body part to the whole body (Herz, 1944). Another 40 years later, Marsden et al. found that an anatomical lesion located in the putamen, caudate nucleus and the thalamus provoked hemidystonia (Goetz et al., 2001; Marsden et al., 1985). This placed dystonia in the movement disorders category.

Epidemiology

According to European statistics the overall prevalence of CD is 58.5 per million, women are more affected (Epidemiological Study of Dystonia in Europe (ESDE) Collaborative Group, 2000). In England, the incidence of CD is estimated at 1.1 in 100.000 inhabitants per year with a prevalence of 18 in 100.00 inhabitants (Butler et al., 2004). As comparison, the incidence of Parkinson's disease is 20.5 per 100.000 with a prevalence estimation of 230 cases per 100.000 (Van Den Eeden et al., 2003). When these estimations are extrapolated to the Netherlands, the

Table 1 - Classification of dystonia

Age of onset	
Early	Before age 26
Late	After age 26
Distribution	
Focal dystonia (single body region)	
	Eyelids: blepharospasm
	Mouth: oromandibular dystonia, musician's cramp
	Larynx: dystonic adductor dysphonia or whispering dysphonia
	Neck: cervical dystonia (spasmodic torticollis)
	Hand and arm: writer's cramp
Segmental dystonia (contiguous regions)	
	Cranial: two or more parts of cranial and neck musculature affected
	Axial: neck and trunk affected
	Brachial: one arm and axial; both arms, with or without neck/ trunk
	Crural: one leg and trunk; both legs with or without trunk
Multifocal dystonia (non-contiguous regions)	
	Hemi: multifocal-ipsilateral arm and leg
Generalized dystonia	
	Leg with trunk and one other region
	Both legs with trunk and one other region
Aetiology	
Primary (or idiopathic) dystonia	
	Sporadic: dystonia is only sign (except tremor) and no cause or degenerative disorder
Secondary (or symptomatic) dystonia	
	Inherited and/ or degenerative disorder
	Acquired or exogenous disorder
	Pseudodystonia (Sandifer's, psychogenic)
	As feature of other neurological disorder (tics, paroxysmal dyskinesia)

Table 1 – Classification of dystonia by age of onset, distribution and aetiology. Adapted from Bressman 2004. Dystonia genotypes, phenotypes and classification. Advances in Neurology: 101-107. Copyright Lippincott, Williams & Wilkins 2004.

population of CD patients is expected to be around 3000 persons compared to 40.000 patients with Parkinson's disease.

Aetiology

Dystonia can be seen as a distinct form of abnormal movement. It is the manifestation of specific impairments in cerebral networks that may result from

various disease entities. This variation is a reason to be careful to extrapolate findings from one entity to another. To classify dystonia, the focus can be on distribution, aetiology or age of onset (see Table 1). CD is the most common form of focal dystonia which is referred to the movement disorders clinic. CD, also called spasmodic torticollis, is an abnormal neck posture combined with neck pain and abnormal head movements with rotation or tilt of the head. Most patients also have jerky movements and forced transient spasms. 20% of patients develop a segmental dystonia with spread of symptoms to lower face, jaw or arm (Weiss et al., 2006).

It is still debated whether the generalized dystonias and the focal dystonias are distinct clinical entities or emerge from the same gene abnormalities with various penetrance. Genetic susceptibility has been proven in generalized dystonia (Nemeth, 2002); it is also likely in focal dystonia. CD patients report that approximately 10% of their relatives also have CD while 26-52% have another form of focal dystonia or essential tremor (Dauer et al., 1998). Exposure to several factors may trigger the development of dystonic symptoms. Various patients report an injury in the neck or the limb prior to the symptoms with incidences ranging from 5-21% (Jankovic, 2001). Also, overactivity of a body part as for example in musicians or writers may be a trigger factor. Animal models in monkeys confirmed that repeated movements of the hand or neck provoke a specific task-induced dystonia (Byl et al., 1996; Evinger, 2005).

Pathophysiology

The pathophysiological mechanisms of focal dystonia are not well understood. After Marsden's discovery of anatomical lesions in hemidystonia (Marsden et al., 1985), others have reported gray matter abnormalities in identical subcortical but also various cortical regions as prefrontal, temporal and sensorimotor cortices in focal dystonia (Egger et al., 2007; Obermann et al., 2007).

Muscle activation and movement related cerebral activation patterns were found to be abnormal. EMG studies disclosed dysfunction of presynaptic reciprocal inhibition. This is present at different levels of the motor system and produces

inhibition of a muscle when its antagonist is contracted; functional impairment would thus lead to co-contraction of muscles and overflow in neighbouring muscles as seen in dystonia (Rothwell et al., 1983). With TMS, reduction of inhibition at a cortical level could be demonstrated together with shortening of the cortical silent period (duration of interruption of voluntary motor activity after TMS) (Siebner et al., 1999a; Stinear and Byblow, 2004). fMRI and PET studies found overactivation in the prefrontal cortex and basal ganglia with underactivation of the primary sensorimotor region and sensorimotor related regions (Blood et al., 2004; Ceballos-Baumann et al., 1995; Oga et al., 2002), although other PET and fMRI studies found the opposite pattern of overactivation in sensorimotor regions (Odergren et al., 1998; Pujol et al., 2000).

Whereas most studies focus on motor control dysfunction, recent studies also underscore the abnormal function of the sensory system. A study in CD mentioned an induction of dystonic symptoms after vibration of the dorsal neck muscles (Lekhel et al., 1997). TMS in writer's cramp has indicated a defective suppression of motor evoked potentials after median nerve stimulation (Abbruzzese et al., 2001) and impaired cortical inhibition and facilitation during muscle vibration (Siggelkow et al., 2002). Sensorimotor impairments were also found during spatial discrimination in writer's cramp patients (Molloy et al., 2003; Sanger et al., 2002). In this respect, it is interesting that many CD patients use sensory tricks, manoeuvres that involve sensory or proprioceptive feedback, to temporarily improve their dystonic symptoms. CD patients usually touch their forehead, cheek or chin to correct dystonic neck turning. Some patients even report an improvement of symptoms while only thinking of their sensory trick (Schramm et al., 2004). Application of the sensory trick in a PET scanner showed that these tricks add extra sensory information which particularly enables antero-ventral parts of the parietal cortex to temporarily switch off the dystonic drive (Naumann et al., 2000).

Most of the results of studies focusing on the pathophysiology of focal dystonia are based on voluntary movement of a dystonic body part. Some studies, however, show that tasks performed by non-dystonic body parts are also abnormal compared to controls. Two studies have documented changes in reciprocal inhibition in both the affected and non-affected arms in writer's cramp and CD

(Chen et al., 1995; Deuschl et al., 1992). Also, CD patients showed reduced muscle recruitment and slower voluntary movement in the upper arm (Carboncini et al., 2004). This would suggest that focal dystonia does not only affect a particular body part, but that it is also present at a subclinical level in all body parts.

Techniques

In this thesis, the following methods have been implemented in order to investigate cerebral activation patterns, structural abnormalities and relations between cerebral and muscle activity: 1) fMRI to identify cerebral activation patterns, 2) TMS to influence cerebral activity, 3) EMG to record muscle activity 4) DTI to investigate anatomical structures.

MRI

The MRI technique is based on the magnetic properties of body tissue (Smith and McCarthy, 1992). It can be used to assess both structure and function of the brain. In clinical practice, so-called structural weighted images are used for identifying anatomical or brain tissue pathology (Symms et al., 2004). However, these images fail to track neural connections between brain areas. A new technique, DTI employs the motion of water molecules in brain tissue (Conturo et al., 1999). These molecules can diffuse freely along a neural fiber but are hindered at fiber crossings, due to the diffusion barrier that is presented by the cell membrane and myelin sheath. By measuring the direction of the diffusion of water molecules in each voxel, DTI can estimate the fiber direction per voxel to create a 3D image of neural fiber orientation. Subsequent fiber tracking is especially useful in brain trauma or acute stroke, but also in diseases that attack the myelin sheath such as multiple sclerosis (Tuch et al., 2003).

Measuring regional brain function requires another strategy. One approach is to employ the relationship between neuronal activity and blood flow. Changes in blood flow are accompanied by changes in oxygen consumption, reflecting the metabolic activity of neurons. However, metabolic changes accompanying brain activation do not follow each other exactly; the local change in cerebral perfusion is much higher than the corresponding oxygen consumption. At synaptic junctions,

excitatory and inhibitory transmissions are equally energy demanding which is reflected by the fact that both are associated with increases of local blood flow. Indeed, the measured signal is hypothesized to reflect astrocyte glucose metabolism or reuptake of neurotransmitters in the synapses (Raichle, 1998). Ogawa et al (1992) were the first to extrapolate this approach to MRI. They made use of a new principle, the blood oxygen level-dependent (BOLD) signal, which is the basis for fMRI. Haemoglobin in red blood cells binds oxygen (oxyhaemoglobin) and changes into deoxyhaemoglobin after oxygen has been delivered to organ tissue such as the brain. Deoxyhaemoglobin sends out a weaker signal than oxyhaemoglobin. Nevertheless, changes in local neuronal activity relate with an increase in BOLD signal, not a decrease. Changes in neuronal activity bring about a higher demand for oxyhaemoglobin. Local vasodilation increases perfusion and raises the oxyhaemoglobin concentration, as it far exceeds the neuronal demand. As a result, venous blood will contain more oxyhaemoglobin than before the change in neuronal activity, so the BOLD signal will increase in that area of brain activation. For the statistical analysis of local changes in BOLD signal, by a voxel-based approach, statistical parametric map (SPM) is a widely used method (Friston et al., 1991). In order to perform the analysis on task-related changes of cerebral activation in groups of subjects, the anatomical dimensions of each brain are spatially normalized onto a standard template (of the Montreal Neurological Institute (MNI)). SPM was first applied to PET results, but became also the most used analysis method for fMRI (Friston KJ et al., 1995).

TMS

TMS is a non-invasive method that can modify brain activity by magnetic stimulation. Since magnetic pulses, in contrast to electric pulses, are not distorted when passing through the skull, they can be used to penetrate the skull and induce an electric current in the brain tissue 2-3 centimetres deep. TMS produces an action potential in the cells that can either have a stimulatory or inhibitory effect on neural networks (Figure 2).

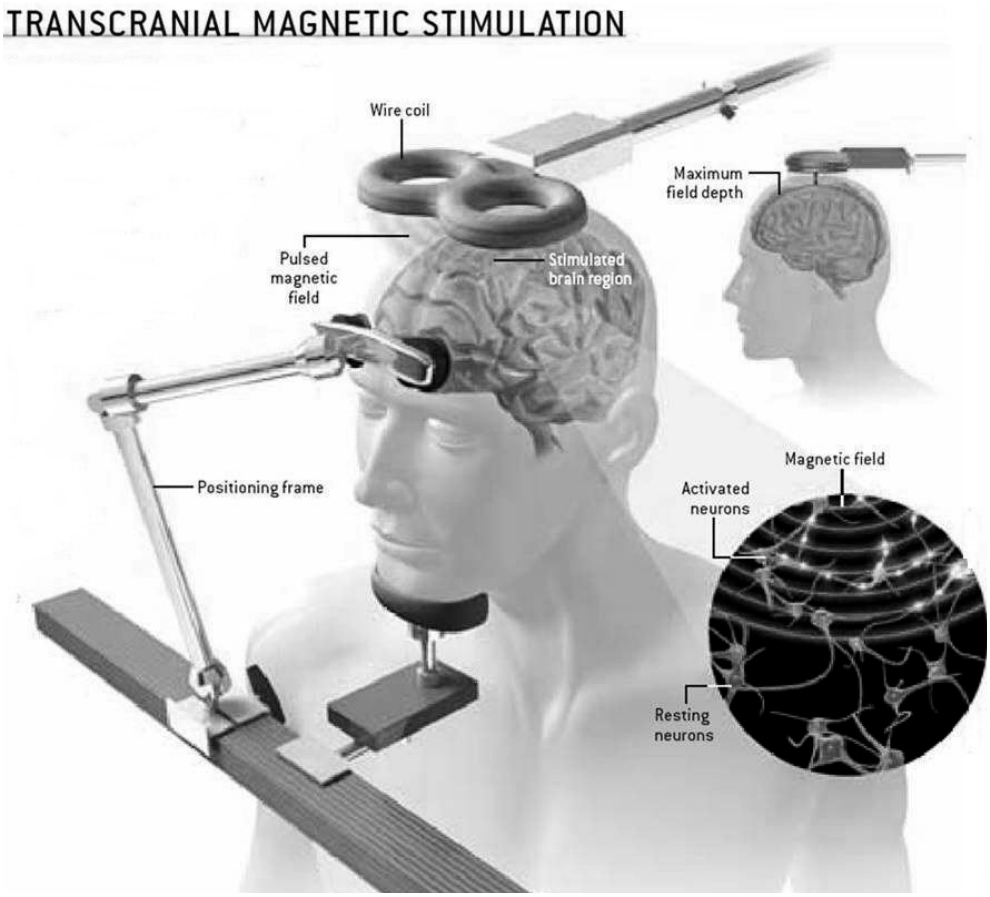

Figure 2 – *TMS employs a coil near the subject's scalp which emits magnetic pulses. These pulses pass safely and painlessly through the skin and bone penetrating the brain a few centimetres deep, lasting microseconds. The magnetic field will then induce an electric current that can activate or inhibit nearby neurons. Adapted from: M.S. George 2003. Stimulating the brain. Scientific American: 66-73. Copyright Bryan Christie Design 2003.*

TMS was first patented by Pollacsek and Beer in 1902 "***to use an electromagnetic coil, placed over the skull, to pass vibrations into the skull and treat depression and neurosis***". However, the TMS equipment that we use today was introduced eighty years later by Anthony Barker (Barker et al., 1985). He described a safe method to directly stimulate the human motor cortex using a pulsed magnetic field. One year later in multiple sclerosis (Barker et al., 1986), TMS was seen as a promising new technique that could be

employed for various applications, ranging from temporarily disrupting the brain to gain insight in fundamental neural networks, to deactivating hyperactive brain regions to stop epileptic seizures.

Nowadays, TMS can be applied in several ways (Kobayashi and Pascual-Leone, 2003). Single pulse TMS is useful in determining motor threshold and central motor conduction times, as Barker did in multiple sclerosis. Motor threshold refers to the lowest TMS intensity necessary to let the target muscle twitch after at least 5 out of 10 TMS pulses. Stimulation above and below the motor threshold is called suprathreshold and subthreshold respectively. Motor threshold provides a good estimate of the excitability of motor neurons in the cortex and spinal cord. Repetitive TMS (rTMS) is the best modulator of cortical excitability. A train of TMS pulses of the same intensity is applied to a brain area at a given frequency. Low-frequency rTMS of 1 Hz or less decreases cerebral cortex excitability, while stimulation with high-frequency TMS of 5 Hz or more increases cortical excitability (Chen, 1997; Pascual-Leone et al., 1998). Supposedly, the modulation effect lasts longer than the duration of the rTMS train. Some studies even reported effect durations up to one hour (Huang et al., 2005). This has been important for the discovery of TMS as a treatment tool for a wide range of neurological disorders ranging from tinnitus (Pridmore et al., 2006) to depression (O' Reardon et al., 2007) and stroke (Mansur et al., 2005). TMS has also been investigated as a treatment option in movement disorders. In Parkinson's disease, 5 Hz rTMS of the prefrontal cortex increased dopamine release in the caudate nucleus which improved reaction time and performance (Pascual-Leone et al., 1994). Subthreshold 1 Hz TMS in writer's cramp patients improved writing reflected in reduced writing pressure (Siebner et al., 1999b).

Recently, new developments have made it possible to combine TMS with neuroimaging. This enables scientists to improve placement of the TMS coil, to understand cortical network connections and to measure the (remote) effects of TMS on the brain by using PET (Fox et al., 1997; Paus et al., 1997) and fMRI (interleaved TMS/fMRI) (Bestmann et al., 2004; Bohning et al., 1998). Although promising, only a few laboratories have managed to solve the technical problems such as synchronization of MRI data acquisition with TMS pulse application,

mounting of the TMS coil in the MRI head coil and minimizing imaging artefacts due to TMS discharge (Bohning et al., 1998). Nowadays, this technique has been used in various studies investigating the (remote) effects of local TMS at either supra- or subthreshold intensity and high or low frequency to M1 (Bestmann et al., 2003; Denslow et al., 2005), prefrontal cortex (Nahas et al., 2001) and also parietal cortex (Kemna and Gembris, 2003; Sack et al., 2007).

EMG

EMG is the study of muscle function by measuring the electrical signal that the muscle emits, as defined by Basmajian in 1985. The signal originates from activity of a motor neuron located in the spinal cord. This neuron generates action potentials that travel along the axon to the neuromuscular junction where they trigger the release of acetylcholine. This neurotransmitter acts on muscle fibers to produce the muscle action potential which in turn results in muscle contraction. With surface EMG, one can record these muscle action potentials by placing electrodes on the belly of a muscle (group) and a ground electrode on a nearby joint or bone. The difference in action potentials picked up by muscle and ground electrodes is reflected in the EMG. For analysis, these recordings can then be rectified (making the values of the signal absolute) and smoothed (reduce the high frequency contents of the signal). This resulting signal can then be analyzed further to determine, for example the amplitude and duration of a muscle contraction.

Outline of the thesis

The main topic of this thesis is the cerebral organization of hand movement in CD, with special attention to the role of the sensory system in the preparation of movement. We first demonstrated with fMRI that in CD abnormalities in cerebral activation patterns occurred during clinically normal hand movement when compared to HC (***chapter 2***). To identify whether such abnormal activation patterns might be related to subclinical abnormalities at muscle level during the clinically normal hand movements, muscle activity was measured during movement performance using EMG (***chapter 3***). Using interleaved TMS/ fMRI, we tried to influence the integration of sensory information for movement preparation in HC by applying TMS to the left superior parietal cortex in order to mimic dystonic activation patterns (***chapter 4***). TMS was similarly used in CD to explore remote cortical effects after disrupting the already impaired parietal function (***chapter 5***). We then investigated whether the impaired cortical function that we found in CD was correlated with structural abnormalities at cortical or subcortical level, as was recently shown in other focal dystonias (***chapter 6***). Finally, we reflected on our studies, the applied paradigms and the obtained results, and noticed that our hand movement paradigm might be further improved to investigate basal ganglia abnormalities in dystonia. Thus, the hand paradigm was further evaluated in the context of initiation and inhibition of movement, since especially inhibition of movement is thought to be impaired in dystonia (Blood et al., 2004). Based on this study we proposed an improved movement paradigm for future studies in CD (***chapter 7***).

Chapter 2

Changed patterns of cerebral activation related to clinically normal hand movement in cervical dystonia

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Abstract

The relief of CD by sensory tricks points at complex sensorimotor interaction. The relation between such stimulus-induced normalization of posture and parietal activation (Naumann et al., 2000) further supports the idea of disturbed higher-order motor control and suggests that the organization of movement is affected beyond the level of a local output channel. Dysbalance beyond a restricted output channel is also supported by the spread of focal dystonia to adjacent body parts. In this fMRI study, we aimed to determine whether CD patients have indeed different patterns of cerebral activation during clinically normal hand performance. By means of SPM of 3T fMRI results, task-related cerebral activations measured in eight CD patients were compared to data of nine HC. Compared to controls, the patient group showed a relative reduction of activations in bilateral parietal, left premotor and cingulate cortex regions during imagery of movement, while activation of right (ipsilateral) putamen, insula and cingulate cortex was impaired during movement execution. CD appears to concern a general disorganization of cerebral motor control, which indicates a pre-dystonic state of clinically normal hand movements. The latter may imply an increased vulnerability for deteriorating triggers such as minor accidents.

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Introduction

Dystonia is a movement disorder characterized by sustained involuntary muscular contractions which cause repetitive twisting movements and abnormal postures of affected body parts (Brin et al., 2004). In CD, this leads to rotation or tilt of the head into a specific direction. Dystonia may remain restricted to an isolated part of the body, although spread to adjacent body parts is not a rare phenomenon (Weiss et al., 2006). Its pathophysiology is not well-known. Functional brain imaging did provide support for the notion that basal ganglia dysfunction, with subsequent abnormal activation of its frontal cortical target regions, plays a key role in this movement disorder (Ceballos-Baumann et al., 1995; Playford et al., 1998). The concept of a primary basal ganglia dysfunction, however, seems incomplete given the apparent role of somatosensory function. Both overt and imagined sensory tricks may alleviate dystonic symptoms (Schramm et al., 2004; Schramm et al., 2007), while sensory signals have been reported to precede the onset of dystonic phenomena. Abnormal activation of somatosensory regions of the parietal cortex further underscores the involvement of disturbed higher-order sensory processing and deficit in sensorimotor integration (Abbruzzese et al., 2001; Leis et al., 1992; Odergren et al., 1996). One might thus infer that sensory tricks add extra sensory information which particularly enables antero-ventral parts of the parietal cortex, that contains the secondary sensory area, to temporarily switch off the dystonic drive (Naumann et al., 2000).

Both basal ganglia and sensory processing theories suggest that during movement execution in dystonia patients an abnormal cerebral network is activated. The fact that a variety of both sensory and task-related conditions influences dystonia may be an argument that such circuitry is not restricted to the cerebral representation of a local body part. In this respect, the role of particularly the parietal cortex, known to participate at a higher level of sensorimotor integration, remains to be further elucidated. To that end, we employed both movement execution and movement imagery tasks in this study. As movement imagery is in the same class of neural processing as movement preparation and planning, neural circuits required prior to movement execution are expected to be activated during movement imagery

(Ehrsson et al., 2003). Moreover, movement imagery enables the study of motor-associated circuitry without the blurring effect of sensory feedback (Stephan et al., 1995). To our knowledge, only one study compared movement imagery with execution in primary dystonia (Ceballos-Baumann et al., 1994). The goal of our study was to determine whether patients with CD have abnormal cerebral activation patterns, particularly in the parietal cortex, while executing and imagining non-dystonic hand movement. To that end, task induced BOLD responses were measured with fMRI in dystonic patients, and compared with the responses obtained in healthy subjects.

Methods

Subjects

Nine HC [age 31-52yrs; 5 males] and eight patients with CD [age 30-55 yrs; 2 males] were studied (Table 1). They gave informed consent to the protocol that was approved by the institutional review board. All were right-handed, assessed by the Annett Handedness Scale (Annett, 1970). Clinically, hand and arm movements were normal. No subject had a medical history of seizures or neurological disorders except primary dystonia. Patients met the formal criteria for dystonia (Brin et al., 2004).

Behavioral task

Subjects performed right-hand tasks in either executive or imagery mode, specified by visually presented words (move, imagine, fist, rest). The hand was held in a vertical plane with the thumb up. Movement execution consisted of a single horizontally directed flexion/ extension movement of the right wrist within a 2-second period. Imagery of flexion/ extension was without overt movements. Subjects had to imagine hand movements as if performed by themselves (first person perspective) (Jeannerod and Decety, 1995). Fist mode execution implied right-hand clenching, which mimicked abundant muscle co-contraction and created a condition with increased intensity of cortical activation (Lotze et al., 1999). During rest, subjects refrained from voluntary movement.

Each condition consisted of a continuously repeated sequence of a 2-second task followed by 2 seconds rest indicated by a cross sign. Subjects had to fill the 2-second task frame with one task option, starting at word presentation and finishing at cross sign appearance. This implied overflow of the task frame into the subsequent 2-second rest frame. Each task was repeated 5 times in one block, followed by a rest block. For each condition, the block was repeated 5 times. Therefore, one task session contained 15 condition blocks (5 blocks per condition), 75 condition presentations (5 condition presentations per block) and 15 rest blocks. The duration of a task session was 10 minutes. Before fMRI, subjects practiced for 5 minutes.

fMRI

HC were scanned at the Medical University of South Carolina, Charleston (USA). CD patients were scanned at the NeuroImaging Center, Groningen (NL). Four HC were additionally scanned in Groningen to compare results with the Charleston HC. One examiner (first author) was present at both locations to guarantee a standardized experimental set-up and subject instructions, and to verify similar task performance in all subjects. At both locations, BOLD fMRI data were obtained with a 3.0 Tesla scanner with standard SENSE coil (Philips, NL) and identical parameters. T2* three-dimensional functional images were acquired with multislice echo planar imaging sequence (EPI). 36 slices (3 mm slice thickness) with 64*64 matrix were acquired as a volume in 1.8 seconds (voxel size 3.5x3.5x3.0 mm). T1-weighted images allowed to define anatomical regions (1.0x1.0x1.0 mm).

fMRI was performed within 60 minutes after task practice. The subject lay supine in the scanner. The head was immobilized and a pillow supported the right arm. This way, the patient had no dystonic posture. In Groningen, visual stimuli were back-projected onto a screen and presented via a mirror built into the head coil. In Charleston, subjects observed a display in front of their eyes. Subjects did not see their hands during scanning.

Analysis

SPM2 (Wellcome Dept. Cognitive Neurology, London) was used for image realignment, transformation into standard stereotactic space (MNI template), smoothing (6x6x6 mm) and statistical analysis. Statistical analysis included an initial threshold of P=0.001 (voxel height response), with cluster size above 30 voxels. CD data were additionally thresholded at P=0.05 (response height), cluster size above 30 voxels. After a general examination of possible activation equivalence between the two groups, a formal between-group analysis was subsequently performed with an initial voxel threshold P=0.001 (extent > 30 voxels). The resulting clusters were regarded significant at P<0.05, corrected for the entire brain volume (cluster-level). The coordinates that indicate the location of local activations are based on adjustment of the Talairach reference system to the MNI standard brain volume (Talairach and Tournoux, 1988).

Results

All subjects performed the task correctly during scanning. Movement rate was similar in the two groups. No overt hand movements were observed during movement imagery. After the experiment, subjects evaluated their performance on a rating scale, with movement imagery as the most difficult task.

Within-group comparisons

In healthy subjects, both execution tasks, flexion/ extension and fist clenching, showed activation of the core elements of movement-related circuitry: the contralateral primary sensorimotor cortex [Brodmann Area (BA) 4,3,1,2] and ipsilateral cerebellum. Both movement conditions further resulted in contralateral thalamus and posterior insula activation. In flexion/ extension movement, parietal cortex activations [BA7,40] were more robust than in fist clenching. On the precentral gyrus, flexion/ extension movement was associated with a wider extension from M1 [BA4] into the premotor cortex [BA6], while fist clenching was related to a more focused activation in M1 [BA4] itself. The coordinates of local

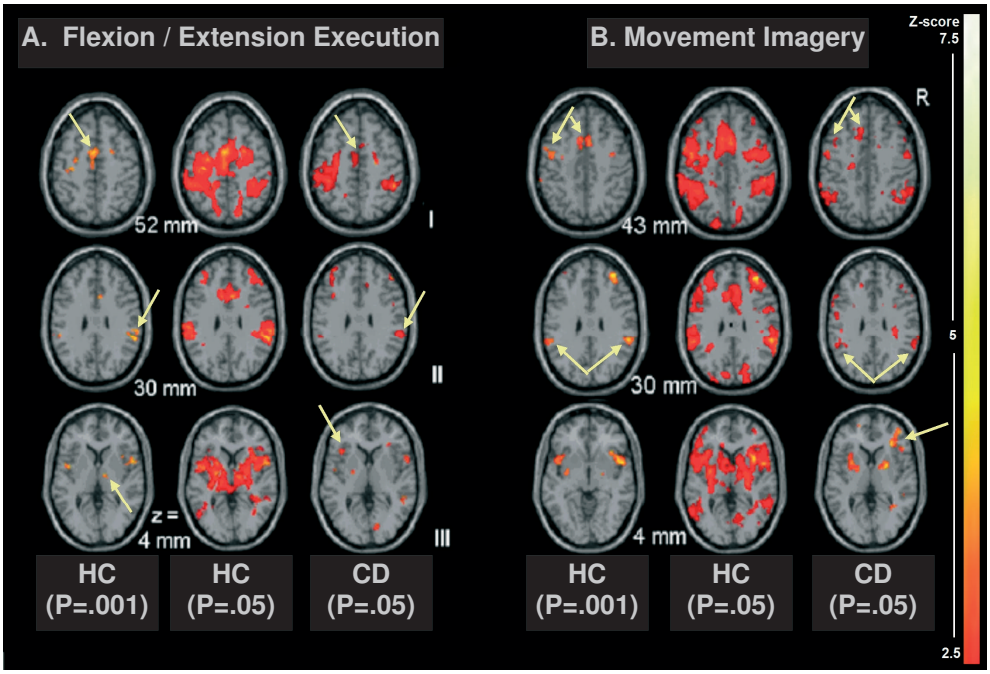

Figure 1 *– Activations during flexion/ extension execution (A) and motor imagery (B) in healthy controls (HC) and cervical dystonia (CD).*

Foci of activation are projected on transverse anatomical MRI slices (T1), made through the standard brain according to the MNI. Slices are parallel to the horizontal section that contains both the anterior- and posterior commissures (z = 0 mm). The distance to this zero-level is indicated.

A. Row I *shows activations around the left central sulcus, thus comprising both the primary motor and primary sensory cortex, in the SMA and bilaterally in parietal and premotor cortex in HC. Only subtle activation of SMA is seen in CD (arrow).* ***Row II*** *presents activation in bilateral inferior parietal cortex with stronger activity on the right side for both HC and CD (arrow).* ***Row III*** *demonstrates low activation of prefrontal cortex in HC; relative stronger activation is seen in CD (arrow). High activation is seen in the posterior insula in HC (arrow), while none is present in CD.*

B. Row I *shows strong left premotor and SMA activation for HC, while weak activation was seen in CD (arrow).* ***Row II*** *presents bilateral activation in the middle frontal, premotor and parietal cortex (arrow) for HC. This activation is decreased in CD.* ***Row III*** *shows bilateral activation in basal ganglia in HC and CD. The right inferior frontal gyrus has been more activated in the CD group (arrow).*

maxima, anatomical locations and statistical Z-scores are listed in Table 2. The results of flexion/ extension movement are further shown in Figure 1A.

Imagery of right-hand flexion/ extension movement by HC showed significant activation in left premotor cortex and SMA [BA6] (Figure 1:B-I). Such activation was

also seen bilaterally in both superior and inferior parietal areas [BA40,7], on the left inferior frontal gyrus [BA44] and the middle frontal gyrus, bilaterally [BA9,10] (Figure 1:B-II). Subcortically, left putamen and right caudate were activated (Figure 1:B-III). No activation was seen in M1 (Table 2, Fig.1B). A decrease in threshold during movement imagery illustrated wider demarcations of bilateral activations in premotor regions including SMA, superior and inferior parietal cortex, inferior frontal gyrus, basal ganglia and insula.

With the same statistical threshold used for HC (initial voxel-level P=0.001), analysis of the CD group resulted only in significant left superior parietal activation during flexion/ extension movement (Table 3). At a relaxed threshold (P=0.05, voxel-level uncorrected), the distribution of activations showed a general resemblance with that in HC, although their extensions were smaller (Fig.1). During the execution tasks in CD, both the premotor cortex [BA6] and M1 [BA4] in the precental gyrus were activated, together with left superior and right inferior parietal cortex [BA7,40] (Figure 1:A-I,II). During imagery, the right inferior frontal gyrus [BA47,44], SMA [BA6] and right inferior parietal cortex [BA40] were activated (Figure 1:B-III,II). Subcortically, bilateral putamen and caudate activations were seen (Figure 1:B-III). Clusters of activation that reached statistical significance after correction for the entire brain volume, are listed in Table 3.

Between-group comparison

For between-group comparison, a two-sided t-test was performed. Results were initially thresholded at voxel-level P=0.001 (uncorrected) with extent minimum of 30 voxels. Resulting clusters were regarded significant at $P<0.05$ (corrected). During task performance, only significant reductions in dystonia-related activations were seen (compared with controls), no significant increases were found in CD.

Comparison between the two groups with regard to the execution of flexion/ extension movements showed a significant reduction of activation in right (ipsilateral) putamen and adjacent insula in the dystonia group (Fig. 2A). Anterior cingulate activation was also reduced, but did not reach statistical significance after brain volume correction (Table 4, Figure 2A). During fist clenching, right putamen

activation was similarly reduced in dystonia. This single difference, however, did not reach statistical significance when corrected for the entire brain volume (Table 4). Relative to controls, imagery of flexion / extension in dystonia was related with significantly reduced activation (P<0.05, brain volume corrected) in right parietal operculum, i.e. S2 (BA40), superior parietal cortex [BA7] bilaterally, left premotor cortex [BA6] and bilateral superior temporal cortex [BA16,38] (Figure 2B, Table 4).

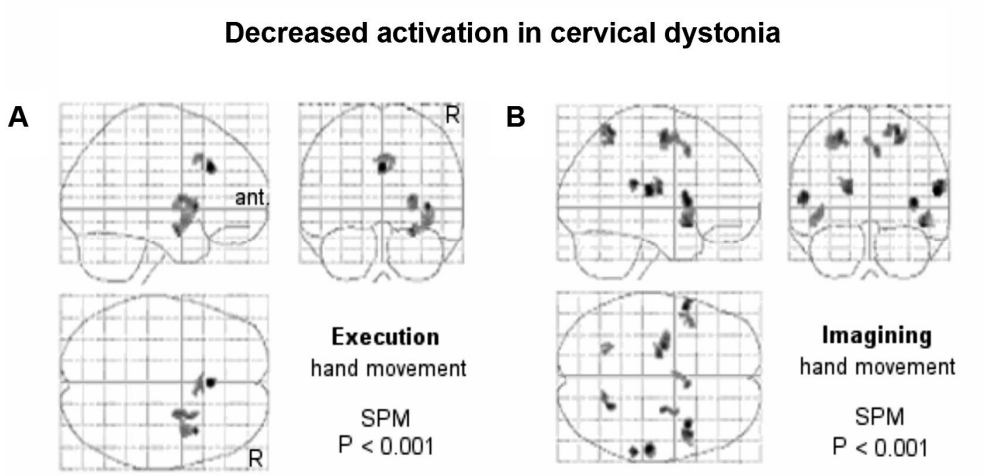

Figure 2 – *Differences in cerebral activation between CD and HC. Activations are projected on a glass brain, with anatomical dimensions according to adjustments of the Talairach reference system to the standard brain of the MNI.*

Panel A *shows regions with impaired activation in CD, compared to HC, during flexion/ extension execution (relative to rest): reduced activation is seen in cingulate gyrus and right putamen and insula.*

Panel B *represents imagery of hand movement with rest: in CD, reduced activation is present in left premotor cortex, ipsilateral postcentral gyrus/ parietal operculum and bilateral superior parietal and superior temporal cortex. See further Table 4.*

Methodological Considerations

The general pattern of activations in CD was lower than in HC, while overt movements were similar. Three possible confounds were considered and subsequently rejected as an explanation of our data. First, possible medication effects in CD, due to benzodiazepines or local botulinum toxin (BTX) injections, were ruled out. By plotting the magnitude of activation for each subject in areas with significant between-group difference, no differences were seen between

patients on and without medication. Second, CD patients were prone to produce movement artifacts. After image realignment, the motion correction plots per subject indeed revealed twice as many head movements in the patients. Comparing individual motion correction plots with individual activation in regions with significant between-group difference did, however, not reveal a relation between head movement and magnitude of local activation. Third, one might argue that the lower average activation intensity in CD was due to differences between scanners. HC were scanned in Charleston (USA) and CD in Groningen (NL). For both scanners, however, manufacturer, magnetic field strength and scan parameters were identical. Moreover, four HC were additionally scanned in Groningen, with results similar to those from Charleston [data not shown].

Discussion

CD was associated with patterns of impaired cerebral activation during both execution and imagery of (normal) right-hand movement. These patterns included parietal cortices, cingulate cortex/ SMA and right (ipsilateral) putamen.

Healthy controls

Activations in HC corresponded with the literature (Lotze et al., 1999). Both execution tasks gave perfusion increases in left M1, bilateral premotor cortex, SMA, cingulate cortex and right cerebellum. Flexion/ extension showed additional bilateral parietal activation, possibly related to the strong spatial characteristics of this task. In M1, the size of activation during flexion/ extension execution was small, while it was large in fist clenching. This is explained by differences in somatotopic representation. Flexion/ extension implies movement of the wrist, which has a small motor cortex representation, whereas clenching involves all fingers, represented over a large field. Moreover, increase of force is related with increased motor cortex activation (Dai et al., 2001).

Imagery of flexion/ extension movement was associated with the activation of regions known for their role in movement preparation: premotor regions including SMA, bilateral parietal cortex, bilateral prefrontal cortex and bilateral cerebellum.

Additional basal ganglia activation was consistent with previous studies (Porro et al., 1996). Movement imagery thus proved to be an adequate strategy for investigating movement preparation without confounds of movement execution (Rodriguez et al., 2004).

Dystonia patients

Although the CD patients reported normal hand movements, associated activation patterns were abnormal. This is consistent with the hypothesis that dystonia is a primary brain disorder expressing itself as one of the focal dystonias (Molloy et al., 2003). The association with abnormal activation during movement in non-affected limbs could explain why focal dystonia can spread to other non-affected body parts, since these might already be in a "pre-dystonic" state. One might speculate that in such a condition, patients are affected by a dormant generalized movement disorder. A mild local trauma or an over-repeated movement may trigger the overt expression of this disorder in the inflicted body part, although often such initial triggers remain unknown.

The association of reduced activation in movement-associated brain regions and clinically normal movement performance implies effective compensation, although subtle changes in movement kinematics cannot be fully excluded. The absence of significant increases of activation in the dystonia group, indeed expected to reflect such compensation, can be explained by individual variation: if a compensation strategy is not embedded in circuitry consistently used by all patients, a significant group result will remain absent. Alternatively, the impaired activations in CD might result from diffuse perfusion increases outside the motor regions, indeed resulting in relative decrease within such regions due to global normalization.

Regional Differences

During imagery of flexion/ extension movement, superior and inferior parietal regions were significantly underactivated in the patients. This may reflect a reduced cortical control of sensorimotor integration as different types of sensory information converge in the parietal cortex. Postero-superior parietal areas provide the entrance of (visuo-)spatial perception, whereas anterior parietal cortex regions are

involved in higher-order somatosensory processing, including the proprioceptic guidance of movement (de Jong et al., 2002). Integration of such information for adequate task performance enables transformation of specific sensory information into information for action (de Jong et al., 2001). The reduced parietal activations in the CD group may thus indicate an impaired integration of the somatosensory consequences of movement in space, leading to a mismatch between representations of body scheme and external space.

In addition to reduced parietal activation, we found reduced medial premotor cortex activation during imagery as well as during execution of movement, the latter was further associated with reduced striatum (putamen) activation. The foci of medial frontal changes comprised the cingulate motor cortex and (pre-)SMA suggesting a reduced efficiency of automatic movement selection (Picard and Strick, 1996; Simon et al., 2002) . Due to their interconnectivity, reduced parietal activation might lead to reduced SMA and premotor activations (Petrides and Pandya, 1984). Low SMA activity has also been found during muscle relaxation and contraction in writer's cramp (Oga et al., 2002). On the other hand, increased activations of (pre-)SMA and prefrontal regions have been found in generalized dystonia patients while executing and imagining freely selected joy-stick movements (Ceballos-Baumann et al., 1995; Playford et al., 1998). This discrepancy might be explained by the strong frontal demand of choice making in free selection. Moreover, our patients had clinically unaffected hands while in the previous studies the tested limbs were dystonic.

Our patients showed significant underactivation of right putamen and insula during movement execution, while overactivation has been a prominent finding in previous dystonia studies (Ibanez et al., 1999; Preibisch et al., 2001). It should be considered, however, that in our study the patients did not have dystonic limbs. It is interesting to notice, in this respect, that we found reduced striatum activations together with medial premotor cortex reductions. During (dynamic) hand immobilization, impaired striatum activation has been found together with increased activations in parietal and cingulate cortices (de Jong et al., 2003). Such immobilization, which implies abnormal sensory feedback, resulted in a temporary clumsy hand. When the loss of overlearned movement was restored after 6-8

weeks, normal striatal activation was found again, while cingulate activation became smaller, thus suggesting regained “automated” movement similar to normal skill acquisition (Van Der Graaf et al., 2004). Beyond their initial compensatory role, one might thus infer that the cortical regions helped to reset the striatum to perform “automated” movements again. In dystonia too, learned motor plans may have lost automatism after receiving abnormal sensory input. However, due to a pre-existing ‘vulnerability’ for dystonia, reflected by the reduced parietal and cingulate activations, insufficient compensation may fail to reset the striatum. On the contrary, abnormal sensory input, such as a minor but painful accident, may act as a deteriorating trigger leading to overt dystonia.

In conclusion, non-dystonic hand movement is associated with abnormal cerebral activation patterns in CD. The combination of reduced striatum activation and both parietal and medial premotor underactivation may reflect a pre-dystonic state in which an abnormal sensory trigger may easily deteriorate normal motor control into a dystonic movement pattern.

Appendix for chapter 2

Table 1 – Cervical dystonia patient characteristics

Subject no.	sex	age (years)	Phenomenology	Illness duration (years)	Treatment
1	F	42	torticollis (R)	30	BTX
2	M	54	torticollis (R) rotation (R)	28	BD
3	F	30	torticollis (L) rotation (L)	4	BTX
4	F	43	torticollis (L) lateroflexion (L)	4	BD
5	F	53	torticollis (R)	16	--
6	F	53	torticollis (L) lateroflexion (L)	20	BD
7	M	55	torticollis (L) rotation (L)	16	--
8	F	52	torticollis (R) lateroflexion (R)	16	BTX

F = female; M = male; L = left; R = right; BD = benzodiazepine (clonazepam); BTX = botulinum toxin. Interval between BTX injection and participation in the study was ½ day for patient 1 and 3 months for patients 3 and 8.

Table 2 Task-related activations in healthy controls

Brain region (Brodmann area)		Left			Z	Right			Z
		x	y	z	score	x	y	z	score
Execution: flexion/ extension wrist									
Frontal lobe	Cingulate g. (BA32)					**0**	**16**	**40**	**4.80***
	Precentral g. (BA4)	**-30**	**-20**	**50**	**4.02***				
Parietal lobe	Inferior (BA40)	**-48**	**-36**	**46**	**3.81***	**60**	**-40**	**32**	**4.71***
	Superior (BA7)	**-12**	**-62**	**64**	**3.53***				
		-22	**-52**	**68**	**3.90***	**10**	**-54**	**68**	**3.89***
Temporal lobe	Superior (BA22)	**-50**	**4**	**-2**	**4.58***	**58**	**8**	**0**	**4.28***
	Thalamus					**12**	**-12**	**2**	**3.79***
	Insula (BA42)	**-44**	**-26**	**18**	**4.46***				
Cerebellum	Posterior	**-22**	**-70**	**-30**	**4.17***				
	Anterior	**0**	**-58**	**-8**	**4.18***	**22**	**-46**	**-24**	**4.59***
		-58	**-34**	**18**	**4.16***				
Execution: fist clenching									
Frontal lobe	Medial (SMA, BA6)	**-4**	**-6**	**54**	**3.95***				
	Precentral g. (BA4)	**-46**	**-14**	**60**	**4.13***				
Parietal lobe	Postcentral g. (BA2)					56	-20	28	4.04
Temporal lobe	Superior (BA22)					**44**	**-2**	**-6**	**3.63***
Subcortical	Thalamus	**-14**	**-22**	**-4**	**4.30***				
	Insula (BA22)	**-40**	**-2**	**10**	**5.25***				
Cerebellum	Anterior					**16**	**-42**	**-36**	**4.68***
Imagery: flexion/ extension wrist									
Frontal lobe	Inferior (BA44)	**-48**	**12**	**12**	**4.60***				
	Middle(BA10/ BA9)	**-34**	**44**	**20**	**4.44***	**36**	**44**	**30**	**4.80***
	Medial (SMA, BA6)	**-6**	**8**	**50**	**5.14***	**26**	**0**	**56**	**4.50***
	Precentral (BA6)	**-30**	**-6**	**48**	**4.85***				
Parietal lobe	Inferior (BA40)	**-56**	**-40**	**32**	**3.93***	**58**	**-38**	**30**	**4.42***
	Superior (BA7)	**-18**	**-60**	**52**	**4.02***	**24**	**-54**	**62**	**3.86***
Temporal lobe	Superior (BA22)					**50**	**8**	**-6**	**4.88***
Subcortical	Caudate Nucleus					**16**	**-2**	**18**	**4.88***
	Putamen	**-30**	**-6**	**12**	**5.08***				
Cerebellum	Posterior	**-38**	**-58**	**-32**	**4.48***				
	Anterior					**22**	**-58**	**-36**	**4.19***

***Note**. Localization of areas with significant increase of BOLD responses by SPM, comparing right wrist flexion/ extension and right fist clenching and right hand imagery of flexion/ extension with rest. Coordinates and Z score of the most significant pixels within each focus of activation are shown. Positive x, y, and z coordinates indicate locations right, anterior and superior of the middle of the anterior commissure. All pixels at P=0.001 and above are presented (with extent threshold of 30 voxels). Coordinates of significant activations (cluster-level P=0.05, entire brain volume correction) are indicated (boldface and *)*

Table 3 Task related activation in cervical dystonia

Brain region (Brodmann area)		Left			Z score	Right			Z score
		x	y	z		x	y	z	
Execution: flexion/ extension wrist									
Frontal lobe	Pre/postcentral gyrus (BA4,6)	-24	-46	66	3.37				
		-24	-4	60	3.12				
Parietal lobe	Superior (BA7)	**-14**	**-52**	**60**	**3.96***				
	Inferior (BA40)					48	-42	52	3.34
Imagery: flexion/ extension wrist									
Frontal lobe	Inferior (BA47,44)					40	36	-6	3.80
	Medial (pre-SMA, BA6)					16	8	52	3.21
Parietal lobe	Inferior (BA40)					44	44	38	2.75

***Note**. Localization of areas with significant increase of BOLD responses by SPM (Z), comparing (i) right wrist flexion/ extension with rest and (ii) imagery of flexion/ extension of the right hand with rest. Initial threshold was at P=0.05 (voxel-level, extent 30 voxels). Only the coordinates in the center of the activations that reach significance at cluster-level after correction for the entire brain volume (P=0.05) are reported in this table. Only the left superior parietal activation reached statistical significance with an initial voxel threshold of P=0.001 (boldface and *). Conventions are as for Table 2.*

Table 4 Reduced task-related activations in cervical dystonia compared to healthy controls (between-group analysis)

Brain region (Brodmann area)		Left			Z	Right			Z
		x	y	z	score	x	y	z	score
Execution: flexion/ extension wrist									
Frontal lobe	Cingulate gyrus (BA32)					0	24	32	4.84
						0	16	40	3.64
Subcortical	Insula					**38**	**12**	**2**	**4.24***
	Putamen					**24**	**0**	**8**	**4.00***
Execution: fist clenching									
Subcortical	Putamen					28	2	-2	4.02
Imagery: flexion/ extension wrist									
Frontal lobe	Middle (BA6)	**-24**	**-10**	**58**	**4.27***	26	-10	56	4.10
	Cingulate gyrus (BA32)					6	10	46	3.92
	Precentral gyrus (BA44)	-56	8	10	4.40				
Parietal lobe	Operculum (S2, BA40)					**56**	**-22**	**14**	**4.84***
	Superior (BA7)					**24**	**-54**	**60**	**4.30***
	Precuneus (BA7)	-20	-60	52	3.72				
Temporal lobe	Superior (BA16/38)	**-44**	**12**	**-6**	**3.64***	**46**	**12**	**-14**	**4.25***
						60	-34	20	4.28
Subcortical	Insula					38	10	6	4.60
	Thalamus	-18	-14	14	4.19				

***Note**. Between-group comparison in HC and CD. CD has a lower task-related increase of BOLD responses. The comparisons presented concern execution and imagery of flexion/ extension movement and fist clenching of the right hand with rest. All pixels at P=0.001 (uncorrected) and above are presented (extent 30 voxels). Coordinates in the center of the activations that reach significance at cluster-level after correction for the entire brain volume (P=0.05) are indicated (boldface and *). Conventions are as for Table 2.*

Chapter 3

Abnormal surface EMG during clinically normal wrist movement in cervical dystonia

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Abstract

We investigated whether patients with CD have abnormal muscle activation in non-dystonic body parts. Eight HC and eight CD patients performed a flexion/ extension movement of the right wrist. Movement execution was recorded by surface EMG from forearm muscles. Although patients had no complaints concerning wrist movement and had no apparent difficulty in executing the task, they demonstrated lower mean EMG amplitude (flexor: 0.32 mV and extensor: 0.61 mV) than controls (flexor: 0.67 mV; P=0.021 and extensor: 1.18 mV; P=0.068; borderline significant). Mean extensor muscle contraction was prolonged in patients (1860 msec) compared to controls (1334 msec; P=0.026). Variation in mean EMG amplitude over movements tended to be higher in patients (flexor: 43% and extensor 35%) than controls (flexor: 34%; P=0.072 and extensor: 26%; P=0.073).These results suggest that CD patients also have abnormal muscle activation in non-dystonic body parts at a subclinical level. This would support the concept that in dystonia, non-dystonic limbs are in a 'pre-dystonic state'.

Acknowledgement

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Introduction

CD is a focal dystonia characterized by sustained involuntary muscular contractions which cause repetitive movements and abnormal postures of the neck and head. In the involved muscles, typically both agonistic and antagonistic muscles co-contract, thus leading to rotation or tilt of the head into a specific direction. EMG recordings of these muscles show long bursts of continuous activity and repeated shorter bursts (Berardelli et al., 1998). In dystonia, abnormalities are not only observed in the pattern of muscle contractions, but also at the level of cerebral activations during preparation (Ceballos-Baumann et al., 1994) and execution of a task in a dystonic part of the body. During task performance, impaired activation in the sensorimotor cortex (Abbruzzese et al., 2001; Leis et al., 1992; Oldergren et al., 1996; Sanger et al., 2001) and SMA (2002; Tempel and Perlmutter, 1993) together with increased activation in prefrontal areas (Ceballos-Baumann et al., 1995; Playford et al., 1998) has been shown in primary dystonia.

These abnormalities in cerebral activation represent disturbances in widely distributed cerebral circuitry, particularly involved in movement planning and preparation. One might thus easily assume that the overt disturbance is not restricted to a single motor outflow channel. The latter is what we generally see in the clinic. Here, focal dystonia expresses itself mostly in one part of the body with a relative risk of spread to adjacent body parts by as yet unknown mechanisms (Weiss et al., 2006).

Recently, a few studies have suggested that non-dystonic body parts might also be affected by dystonia but at a subclinical level (Siggelkow et al., 2002; Thickbroom et al., 2003). In a neurophysiological study, Carboncini et al. have used EMG and kinematic variables to investigate horizontal arm extension movements in patients with CD. These authors have reported impaired recruitment during the initial phase of movement in the clinically unaffected deltoid muscle (Carboncini et al., 2004). In a neuroimaging study which we recently performed, cortical activation was investigated during movement execution and imagery of the clinically normal, non-dystonic right wrist in CD. Compared to HC, CD patients showed reduced activation in the superior parietal cortex and medial- dorsolateral prefrontal cortex

and increased activation in the ventral prefrontal cortex during movement imagery with additional impaired activation in putamen, insula and cingulate cortex during movement execution (***chapter 2***).

To further investigate this concept, we have set up an EMG study along the research line of our neuroimaging study. We similarly employed EMG recordings as in the study of Carboncini et al.(Carboncini et al., 2004). However, we extracted additional variables from the EMG and compared these between patients and HC. In addition, we investigated distal muscles, assuring that activation of the clinically dystonic muscles will not directly influence the EMG recordings in non-dystonic muscles.

The aim of this study is to determine whether patients with CD exhibit not only cerebral changes but also abnormalities in muscle activation patterns during movement of a clinically normal, non-dystonic part of the body.

Methods

Subjects

Eight HC [mean age 50 +/- 8 (standard deviation (SD)) years; 4 women] and eight patients with CD [mean age 48 +/- 10 (SD) years; 7 women] participated in this study after giving informed consent (see Table 1 for subject details). The study was approved by the local institutional review board. All subjects were right-handed as assessed by the Annett Handedness Scale (Annett, 1970). No subject had a medical history of neurological disorders other than primary dystonia. On the same day as the experiment, an independent neurologist screened the patients to determine the extent of their dystonia and to exclude patients with dystonic symptoms in the right hand. Medication was continued as usual during the study (Table 1).

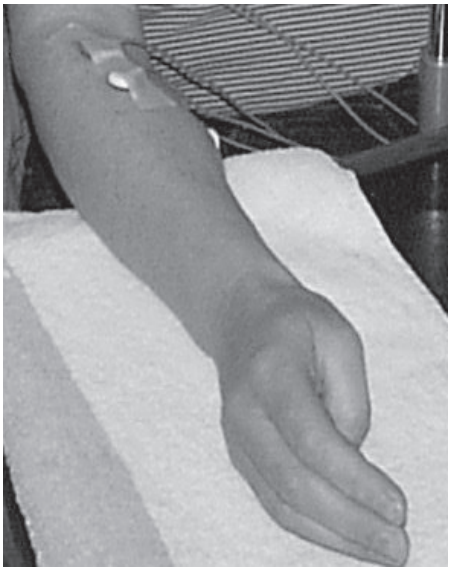

Figure 1 *– Picture demonstrating hand position in the neutral position during rest. The hand is in the vertical plane with thumb up and fingers touching each other. Flexion/ extension of the wrist is performed by moving the hand first laterally inward and then outward.*

Experimental setup

The subjects were studied while sitting in a comfortable chair, with their chin in a rest. To restrict head movement, a band was strapped around the subject's head and fixed to the chin rest. All subjects performed the task with their right hand which rested on a padded platform. Visual stimuli (move or +), each presented for 2 seconds, were shown on a computer screen and specified the performance mode. During the 'move' condition, the subjects executed one lateral flexion/ extension movement of the right wrist, with the fingers aligned with each other and thumb up in the vertical plane (Figure 1). The subjects were instructed to perform the movement smoothly from the neutral to the most flexed, to the most extended and finally to the neutral position again. Every condition consisted of one movement execution followed by rest, indicated by the cross sign. Subjects had to fill the 2-second period as completely as possible with the instructed condition, starting the flexion/ extension movement at first presentation of the word 'move' and stopping when the cross sign appeared. Each condition was repeated 5 times in one segment; each segment was repeated 5 times in one run (Figure 2). Before the experiment started, subjects performed one practice run. During the experiment, the subjects underwent 6 runs with the same conditions. Continuous video monitoring was used to assess task performance to ensure that all

movements were performed correctly and within the set time frame. To prevent fatigue, subjects rested for 5 minutes between runs.

Movement performance was quantified by bipolar EMG, which was recorded from right flexor carpi radialis and extensor carpi radialis longus muscles. Two Ag/AgCl surface electrodes (Neotrode) were placed 1 cm apart on the belly of each muscle. The EMG was digitally recorded using Brainlab 4 (OSG, Rumst, Belgium). Sampling rate was set at 1000 Hz and a band-pass filter of 10-350 Hz was applied.

Analysis

EMG analysis was performed using Brain Vision Analyzer (v. 1.05, Brain Products GmbH, Munich, Germany). The obtained data was filtered with a moving average filter (100 data points window) and rectified. Next, the data was segmented into five

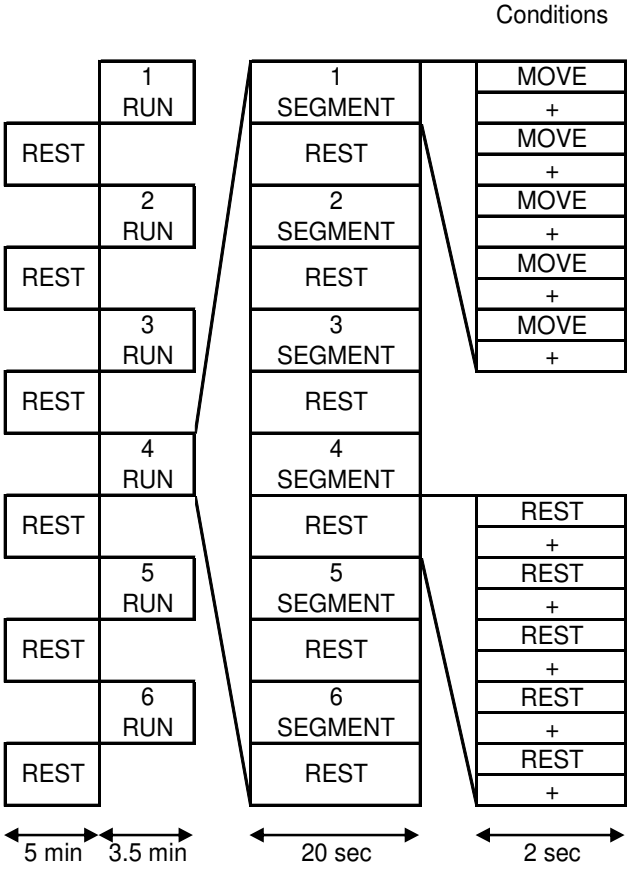

Figure 2 – *Schematic overview of the experimental set-up. The experiment consisted of 6 runs. Each run consisted of 5 segments, each segment containing 5 condition blocks containing either the movement condition (move) or the rest condition (+).*

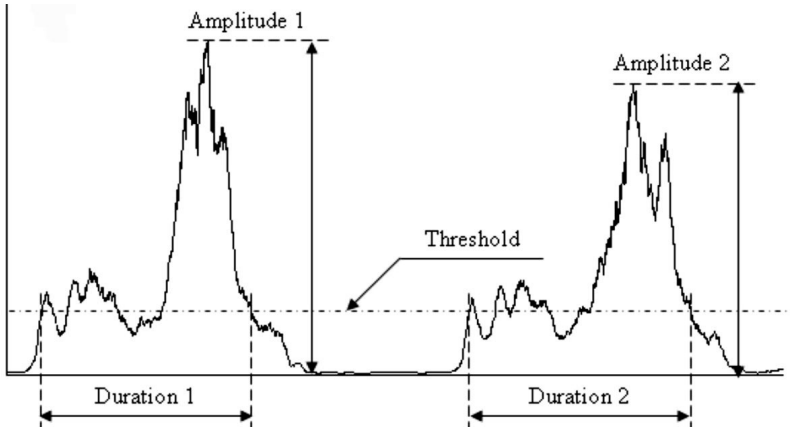

Figure 3 - *Smoothed and rectified EMG data of two EMG bursts. The bursts are delineated using a threshold of 140% of mean overall amplitude. For further analysis, the duration is determined as the period between the first and last time point at which EMG crosses the threshold.*

movement condition blocks, each containing five EMG bursts. Further analysis was done in Excel. The on- and offsets of the EMG bursts were selected automatically, using a threshold of 140% of the mean overall amplitude (Figure 3). This threshold was determined so that automatically defined bursts visually coincided with manually defined bursts. Three quantative EMG variables were derived for each run: the mean duration of EMG bursts (duration), the mean amplitude of EMG bursts (amplitude) and the variation in mean amplitudes of EMG bursts (range). The variable range provides a measure of constancy of task performance over runs. The duration and amplitude of EMG bursts were calculated per 'move' condition block and per run. The range was calculated by comparing the maximum mean amplitude and minimum mean amplitude relative to the minimum amplitude ((max-min)/min) over the five movement blocks per run.

Statistical analysis was performed in SPSS 10. Repeated measures Analysis of variance (ANOVA) were performed for all six EMG variables (duration, amplitude and range for both flexor and extensor muscles). This analysis allows to take into account the within subject variability, resulting from multiple repetitions of the same task. The within subjects factor, with the EMG variable as the dependent variable, was run number (6 levels: 1-6). The between subjects factor was group category (controls or patients). In addition, to evaluate possible interactions between group

and muscle, a factorial ANOVA was performed on all variables averaged over runs, using group category as between subject and muscle (flexor or extensor) as within subject factor. $P<0.05$ was assumed significant.

Results

All subjects performed the task correctly. No overt difference in movement execution between and within groups was seen. Subjectively, neither controls nor patients reported fatigue during the experiment.

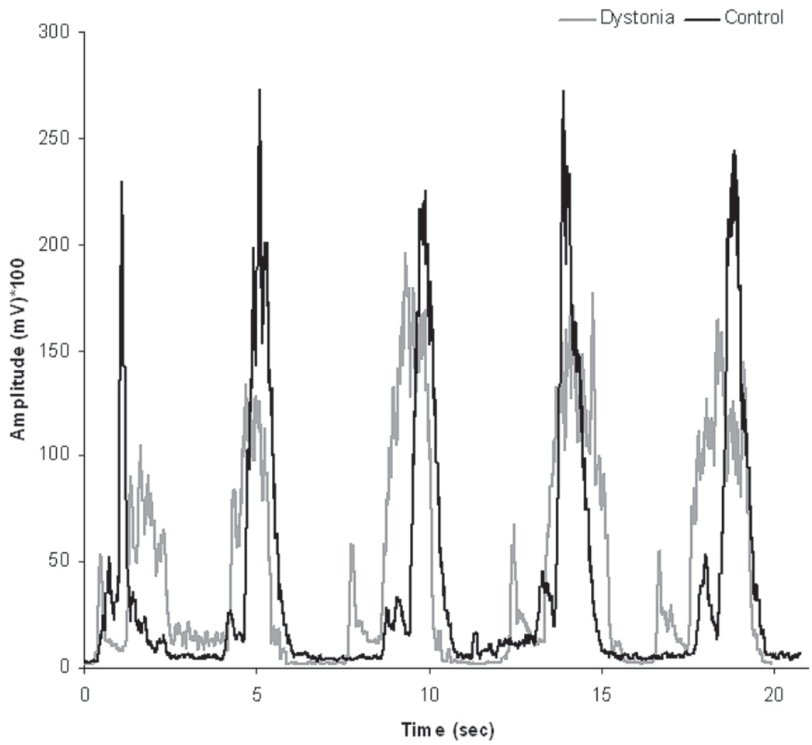

Figure 4 *- Smoothed and rectified EMG data. One segment of extensor EMG data in a control (black line) and a patient with CD (grey line) at the same time scale. The patient clearly shows a longer duration of contractions together with lower amplitudes. In addition, the amplitudes of the EMG bursts are more variable.*

EMG data

DURATION. The mean duration over all runs was compared between groups. In HC, flexor duration was longer than extensor duration, while CD patients showed longer extensor than flexor duration (HC: flexor 1598±49 msec (mean± standard error), extensor 1334±47 msec; CD: flexor 1668±61 msec; extensor 1860±80 msec). A clear difference was seen for extensor duration between groups (P=0.026) while this difference was not so clear for flexor duration (P=0.692) (Tables 2-3, Figures 4-5). Nevertheless, the factorial ANOVA indicated no significant main (muscle: P=0.900, group P=0.415) or interaction (group by muscle: P=0.108) effects.

AMPLITUDE. Both controls and patients had lower flexor amplitudes than extensor amplitudes (HC: flexor 0.67±0.05 mV and extensor 1.18±0.10 mV; CD: flexor 0.32±0.02 mV and extensor 0.61±0.05 mV). For both muscles, however, the amplitudes were lower in patients than in controls, although for extensor muscles this difference was only borderline significant (flexor P=0.021; extensor P=0.068) (Tables 2-3, Figures 4-5). These differences did not result in significant main (muscle: P=0.168, group P=0.149) or interaction (group by muscle: P=0.495) effects in the factorial ANOVA.

RANGE. Flexor range was higher than extensor range in both groups. [HC: flexor 34±3 %; extensor 26±2 %; CD: flexor 43±4 %, extensor 35±3 %]. Yet, the range tended to be lower in controls than in patients for both muscles (flexor: P=0.072; extensor: P=0.073) (Tables 2-3, Figures 4-5). The factorial ANOVA confirmed these results by showing significant main effects for muscle (P=0.012) and for group (P=0.010), but no interaction effects (group by muscle: P=0.966).

In the repeated measures ANOVA, there was no main effect for run or interaction between run and group for any of the EMG variables. Also, no overlap of importance of flexor and extensor muscle contractions, as visually evaluated using Brain Vision Analyzer, was seen during the hand movement in patients, thus providing evidence for the clinical absence of dystonia in the arms.

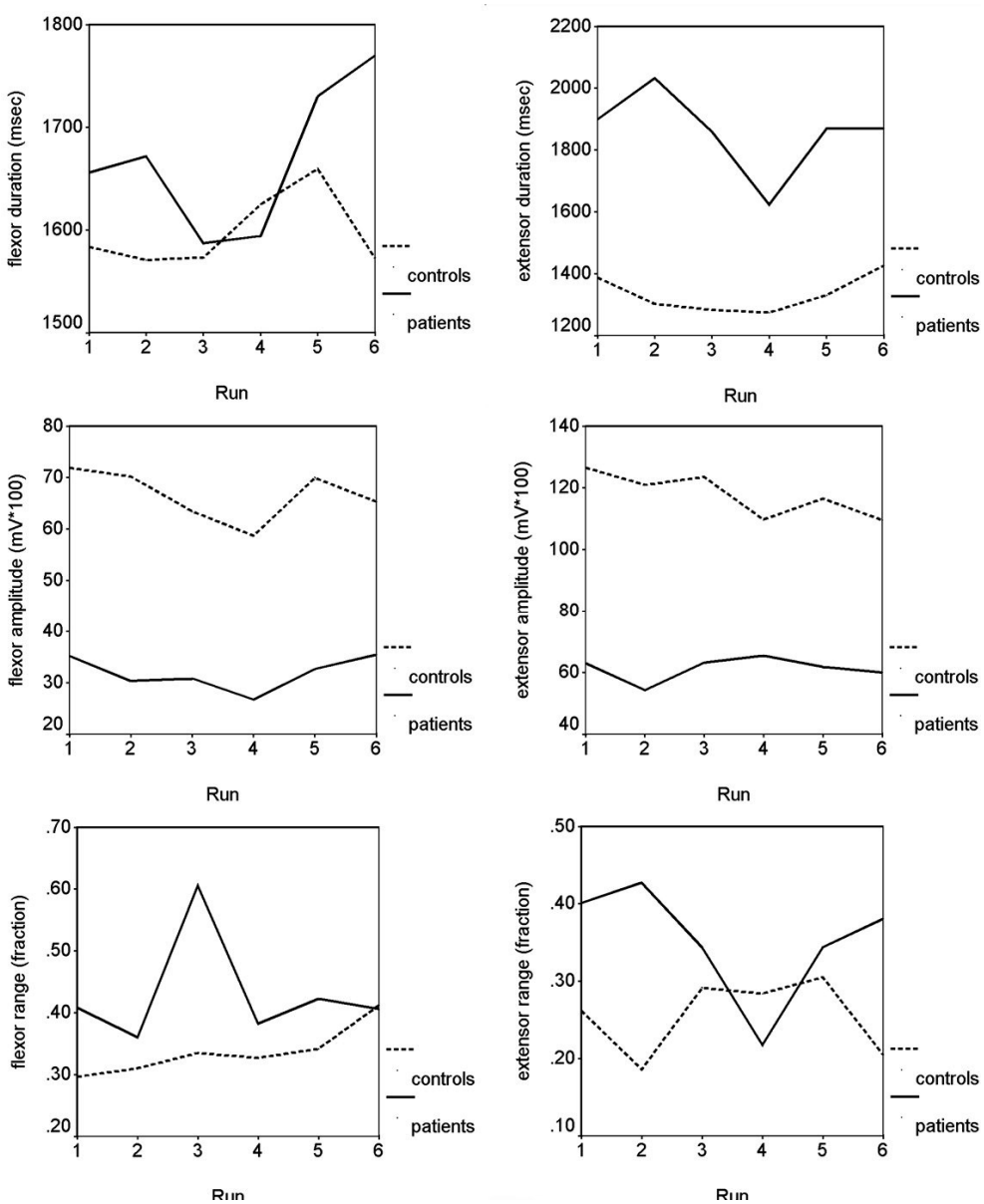

Figure 5 *- Mean values per run for duration (top), amplitude (middle) and range (bottom) for flexor (left) and extensor EMG (right). Drawn line: patients with CD; dashed line: healthy controls.*

Discussion

We demonstrate here that an abnormal EMG pattern is present during wrist movement execution of a clinically normal, non-dystonic hand in patients with CD. This pattern mainly consists of lower mean flexor EMG burst amplitude together with prolonged extensor EMG burst duration.

Clinically abnormal EMG patterns during voluntary movement of a dystonic limb have been well-described. In their review article, Berardelli et al. described slowness of voluntary movement in dystonic body parts and prolongation of time needed to switch between movements. Additionally, lack of muscle selectivity may be seen when attempting to perform a movement, leading to an overflow of activity to muscle groups not normally activated in the movement. EMG bursts are often prolonged, which may result in overlap of agonist and antagonist muscles for a longer period of time than normal (co-contraction) (Berardelli et al., 1998).

Although these findings are based on voluntary movement in dystonic body parts, other studies share our assumption that movement in non-dystonic body parts might also be abnormal, but on a subclinical level. Two studies have reported similar changes in reciprocal inhibition in affected and unaffected arms of writer's cramp patients and in unaffected arms of CD patients (Chen et al., 1995; Deuschl et al., 1992). Likewise, CD patients without overt dystonic involvement of upper arm muscles showed reduction in agonist muscle recruitment during arm extension, with slower voluntary arm movement (Carboncini et al., 2004). It has been suggested that impairments also occur on sensorimotor level due to altered control of proprioceptive input in the hand in CD patients (Siggelkow et al., 2002).

The studies mentioned above and our results fit a general hypothesis: dystonia patients have a primary disorder in the brain that may express itself, by largely unknown mechanisms, as one of the focal dystonias (Molloy et al., 2003). Although dystonic involvement is clinically only seen in one part of the body, other parts of the body may exhibit subclinical dystonic movement abnormalities. This concept could be labeled as 'pre-dystonic state'. It is substantiated by the less effective muscle activation – consisting of longer duration of extensor muscle contraction and lower amplitude of flexor muscle contraction - that we observe in our patient

group. Interestingly, no clear overlap of flexor and extensor muscle contractions (co-contractions) were seen in this study, although they are a typical symptom of dystonia. It may be that the progression from a pre-dystonic into a dystonic movement goes through different phases in which different dystonic symptoms occur. Co-contraction might then be a symptom occurring in a later phase. Although we attribute the quantative changes of EMG to the disease condition, the influence of treatment needs to be addressed. Half of our patients were treated with BTX injections in the neck. BTX acts presynaptically by inhibiting the release of the neurotransmitter acetylcholine. This results in a temporary paralysis and, if chronically injected, atrophy of a part of the muscle fibers (Hamjian and Walker, 1994). In dystonic muscles, BTX is used as treatment to decrease the muscle force and thus temporarily “normalize” the muscle imbalance (Gilio et al., 2000). Although there are distant effects of locally applied BTX, the relative importance is not clear. Some studies investigated CD patients in which chronic treatment of BTX in the neck resulted in histologically determined atrophy in leg muscles without clinical symptoms (Ansved et al., 1997) or discrete changes in muscle fiber conduction velocity based on both muscle atrophy and hypertrophy possibly to compensate denervation [Lange et al: personal communication]. In contrast, other studies demonstrate that there is no (distant) leg muscle atrophy (Fertl et al., 2000) or subclinical muscle weakness (Olney et al., 1988). In addition, it has been reported that BTX treatment in CD may even normalize the displaced topography of corticomotor projection of the (clinically non-dystonic) abductor pollicis brevis muscle (Thickbroom et al., 2003). These results support our assumption that BTX treatment in CD may have slight distant effects on the flexor and extensor muscles, but that these effects tend to normalize muscle function. If we focus on our study, 4 patients were treated with BTX injections. There were no significant differences between the treated and untreated patients for all tested EMG variables (Table 3).

In conclusion, this study demonstrates that abnormal EMG patterns are present during clinically normal movement in a non-dystonic part of the body in patients with CD. These findings provide support for the assumption that dystonia not only affects the clinically manifest parts of the body, but that the whole body is in a pre-dystonic state.

Appendix for chapter 3

Table 1 - Subject characteristics

Subject no.	Sex	Age (years)	Type of torticollis (direction)	Illness duration (years)	Treatment*
CD					
1	M	54	rotation (R)	28	BD
2	F	53	rotation, anteflexion (R)	16	--
3	F	30	rotation (L)	4	BTX
4	F	52	lateroflexion (R)	16	BTX
5	F	53	lateroflexion (L)	20	BD
6	F	48	anteflexion (R)	5	BTX
7	F	49	rotation (L)	7	--
8	F	50	rotation (R)	7	BTX
Mean ± SD		**48.6 ± 7.8**			
HC					
1	F	42			
2	F	49			
3	F	58			
4	M	27			
5	M	52			
6	M	54			
7	M	44			
8	F	54			
Mean ± SD		**47.5 ± 9.8**			

CD = cervical dystonia patient; HC = healthy control; F = female; M = male; L = left; R = right; BD= benzodiazepine (clonazepam); BTX = botulinum toxin SD = standard deviation

** Interval between BTX injection and participation in the study was 2 weeks for patient 3 and 3 months for patients 4, 6, 8*

Table 2 - Mean value per EMG variable per run in healthy controls and cervical dystonia patients

	Duration (msec)				Amplitude (mV)				Range (%)			
	HC		CD		HC		CD		HC		CD	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
Flexor												
Run 1	1584	107	1656	136	0.72	0.14	0.35	0.06	30	5	41	7
Run 2	1571	104	1672	142	0.70	0.14	0.30	0.06	31	4	36	6
Run 3	1573	113	1587	163	0.63	0.11	0.31	0.04	34	6	61	16
Run 4	1625	111	1594	146	0.59	0.10	0.27	0.03	33	10	38	7
Run 5	1660	156	1731	161	0.70	0.17	0.33	0.08	34	9	42	7
Run 6	1573	156	1770	190	0.65	0.11	0.35	0.09	41	6	41	4
Overall mean	**1598**	**49**	**1668**	**61**	**0.67****	**0.05**	**0.32**	**0.02**	**34***	**3**	**43**	**4**
Extensor												
Run 1	1387	133	1900	225	1.27	0.34	0.63	0.14	26	4	40	6
Run 2	1303	123	2033	213	1.21	0.30	0.54	0.13	19	2	43	7
Run 3	1283	102	1859	171	1.24	0.28	0.63	0.13	29	5	34	9
Run 4	1274	79	1624	170	1.10	0.19	0.65	0.13	28	7	22	4
Run 5	1331	133	1870	223	1.17	0.23	0.62	0.13	31	9	34	8
Run 6	1426	143	1871	206	1.10	0.24	0.60	0.11	21	3	38	7
Overall mean	**1334****	**47**	**1860**	**80**	**1.18***	**0.10**	**0.61**	**0.05**	**26***	**2**	**35**	**3**

*CD = cervical dystonia patient; HC = healthy control; SE = standard error; ** = P<0.05 and * = 0.05<P<0.10 as resulting from repeated measures ANOVA, CD compared to HC*

Table 3 - Average value over all runs per EMG variable and muscle for each subject

Subject	Flexor Duration (msec)	Flexor Amplitude (mV)	Flexor Range (%)	Extensor Duration (msec)	Extensor Amplitude (mV)	Extensor Range (%)
HC 1	1480	0.29	22	1256	1.31	17
HC 2	1858	0.56	35	1730	0.70	19
HC 3	1171	0.81	39	1496	0.96	20
HC 4	1297	0.92	48	1061	2.23	48
HC 5	1459	0.35	26	1173	0.63	33
HC 6	2000	0.28	27	1627	0.34	27
HC 7	1495	0.90	47	867	2.31	19
HC 8	2021	1.22	25	1464	0.95	22
CD 1	1258	0.24	39	1249	0.45	48
CD 2	1742	0.43	45	2216	1.09	20
CD 3	1803	0.12	56	2476	0.13	42
CD 4	1208	0.22	44	1686	0.18	24
CD 5	1288	0.61	48	1156	0.65	43
CD 6	2180	0.33	50	1533	0.91	35
CD 7	1812	0.25	35	2199	0.95	33
CD 8	2056	0.36	28	2363	0.55	36

CD = cervical dystonia patient; HC = healthy control; grey: BTX-treated patients

Chapter 4

Changes in cerebral activations during movement execution and imagery after parietal cortex TMS interleaved with 3T MRI

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John A. Walker, Mark S. George, Klaus L. Leenders

Submitted

Abstract

The left parietal cortex contributes to goal-directed hand movement. In this study, we targeted this region with TMS to assess the effects on a wider distributed circuitry related to motor control. Ten healthy subjects underwent 3 Tesla MRI with interleaved TMS. They either executed or imagined right wrist flexion/ extension movements, which was preceded by a 10-second period either with or without TMS. This was applied to the left superior parietal cortex in 10 stimuli of 1 Hz at 115% motor threshold intensity. TMS in the movement execution condition resulted in significantly increased activation in the bilateral prefrontal, right temporo-parietal and left posterior parietal cortices, when compared to movement without such intervention ($P<0.001$ voxel-level; $P<0.05$, volume corrected). Movement imagery with TMS showed significantly increased activation in the left medial prefrontal cortex, right lateral prefrontal cortex, left supramarginal gyrus and right occipital cortex, while a decrease was present in bilateral anterior parietal cortex ($P<0.01$ voxel-level; $P<0.05$ volume corrected). TMS-induced activation changes of left superior parietal cortex thus appears to increase prefrontal and posterior parietal cortex activation, associated with a reduced function of the anterior parietal cortex, including S2. These changes are thought to reflect an impaired ability to estimate the proprioceptive consequences of movement during its preparation, which is compensated by the increased contribution of more remote parietal and prefrontal cortical regions.

Acknowledgement

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Introduction

Interleaved TMS within the fMRI scanner is a new technique for brain activation studies. It allows the researcher to stimulate and simultaneously measure TMS-induced changes in activation of neural circuits by monitoring changes in BOLD responses (Bohning et al., 1998). It has successfully been implemented in studies mainly investigating primary motor and prefrontal cortex (Bestmann et al., 2003; Nahas et al., 2001), although some studies have targeted the parietal cortex. Kemna and colleagues (2003) performed posterior parietal TMS in resting state, which did not result in changed activation. In a visuomotor task, right (but not left) superior parietal TMS resulted in both impaired visuospatial judgement and changed activations in fronto-parietal circuits (Sack et al., 2007). In the present study, we modulated parietal cortex function with TMS to investigate the effects on circuitry involved in the organization of simple proprioception-dominated movements. The parietal contribution to higher-order motor control (Georgopoulos, 1991) includes the integration of visuospatial and somatosensory information to generate accurate commands for action (Binkofski et al., 1999; Castiello, 2005; de Jong et al., 2001). Parietal lesions may result in motor symptoms such as ideomotor apraxia (Poizner et al., 1995) and optic ataxia (Battaglia-Mayer and Caminiti, 2002), while experimental impairment by TMS induces transient disruption of adjustments of reaching movements (Desmurget et al., 1999) and grasping (Rice et al., 2006).

A role of the parietal cortex in sensorimotor integration at the level of simple movements can be inferred from functional imaging in healthy subjects (Stenekes et al., 2006) and research addressing a movement disorder such as dystonia (Naumann et al., 2000). One of the features of simple movements, in contrast to complex visuomotor tasks, is that simple movements require less elaborate processing of visual information in the posterior part of the parietal cortex (Deiber et al., 1996; Sadato et al., 1996). In cerebral activation studies, with global normalization, such movements without visual feedback reveal relatively stronger activations related to proprioceptive processing, in which anterior parietal regions play a role (de Jong et al., 2002; Ehrsson et al., 2001; Hinkley et al., 2007; Naito et

al., 2005). TMS-induced changes in the parietal cortex during simple movement tasks may therefore add insight into the parietal role in complex movement tasks.

Recently, we have demonstrated that imagery of simple hand movements in CD patients was related with reduced activation in the superior parietal cortex and S2 (***chapter 2***). That finding supported the concept that in dystonia an impaired integration of proprioceptive feedback and spatial movement planning implies a mismatch between representations in body scheme and external space that leads to distorted movements (de Jong et al., 2001; Naumann et al., 2000). In the present study, the left superior parietal cortex was targeted with TMS to test the hypothesis that local functional impairment might result in a decrease of task-related activations, both at the stimulus site and in the connected parietal-premotor regions, thus mimicking the changes observed in dystonia. Moreover, we expected increased activations in prefrontal areas similar to the 'compensatory' increases in dystonia patients. Alike the dystonia study, we employed a robust movement imagery task (***chapter 2***)(Gieteling et al., 2008) enabling the assessment of circuitry for movement preparation without blurring effects of actual sensory feedback that accompanies movement execution (Stephan et al., 1995). This may provide information to what extent circuitry dealing with somatosensory-motor transformations contributes to movement preparation (Christensen et al., 2007; McCloskey, 1981).

Methods

Subjects

Ten HC (mean age 53 +/- 11 (SD); 8 females) were studied after having given written informed consent approved by the Medical University of South Carolina institutional review board. All were right-handed, assessed by the Annett Handedness Scale (Annett, 1970). No subject had a medical history of seizures or neurological disorders. Subjects were included for fMRI when they were able to perform the instructed tasks as explained in the following paragraph.

Behavioral task

Subjects performed both movement execution and imagery tasks to assess activation changes in circuitry supporting higher-order motor control, induced by TMS at the parietal cortex. Although movement imagery has been used before in neuroimaging studies (Hanakawa et al., 2003), it remains difficult to check if and how subjects perform the task inside an MRI scanner. Some studies chose to connect a behavioral component to the imagery task such as mental rotation or handedness recognition (Wilson and Farah, 2006). The set-up used in our study did not permit a projection screen inside the MRI scanner with visualization of such a behavioral component. However, we determined the imagery performance of subjects with a questionnaire and timing test during a visit to the clinic a few weeks prior to the start of the experiment. The ability of subjects to perform and imagine the task was checked with a Vividness of Movement Imagery Questionnaire (Isaac et al., 1986). Two self-report lists evaluate the ability to imagine several movements as done by themselves (first-person perspective) or others (third-person perspective) (Jeannerod and Decety, 1995). The questionnaire uses a scale from 1-5 (vivid to no imagination) to score two lists of 24 items, the total scores thus range from 48-240. In our subject group, the subjects who were included in the experiment had a mean score (± SD) of 109 ± 58. This average score was similar to scores reported in previous studies (Gieteling et al., 2008; Williams et al., 1995). In addition, two cycles of 10 movement executions and 10 imagery movements were timed. Subjects were included for fMRI when they had a good imagery performance (<1.5x the time of an execution cycle). Their speed of performance in the movement imagery and execution tasks was 17s ± 7 and 23s ± 13, respectively (mean ± SD).

Behavioral task during fMRI

After inclusion, each subject underwent one 15-minute session during which the effect of interleaved TMS/fMRI was assessed. During the fMRI scan, subjects performed right-hand tasks successively in executive, imagery or rest mode, specified by visually presented LED lights: the green light alerted the subject to

move, the red light specified imagine and no light specified rest. Each of these tasks was performed either with or without preceding TMS. Movement execution consisted of a flexion/ extension movement of the right wrist in a horizontal plane. Imagining flexion/ extension was without overt movements: subjects had to imagine performing the flexion/ extension movement of the right wrist as if it was performed by themselves (first person perspective). During rest, subjects refrained from voluntary movement. The hand was held in an armrest for support while the fingers were holding a vertical handle mounted to a short arm that pivoted just under the wrist. The extent of the movement execution was registered by recording the movement of the handle. This registration was also used to check if subjects indeed refrained from movement during the imagery task and rest period. The sensor in the arm rest showed that all subjects performed the task correctly. No overt hand movements were observed during movement imagery. Before the experiment started, subjects practiced the tasks for 5 minutes. The difficulty of the tasks was scored by the subjects on a 1-5 scale (impossible – normal). These ratings were requested at three time points: before the scan during the practice session, right after the scan to determine their performance during scanning and 30 minutes after the scan during the evaluation of the experiment.

The fMRI paradigm was designed as follows: one run contained 2 blocks of the same task, each block started with either a train of 10 TMS pulses or 10 seconds without TMS. Each block contained 5 performance cycles with an identical 2-second task frame followed by a 2-second pause frame (Figure 1). Subjects had to fill the 2-second task frame with one task option, starting at the LED light presentation and finishing at disappearance of the light. Each task option (execution, imagine or rest) was repeated in 5 runs, with a total of 15 runs. The duration of one run was 60 seconds so the total duration of an fMRI session was 15 minutes. The conditions were ordered in a fixed randomized setting.

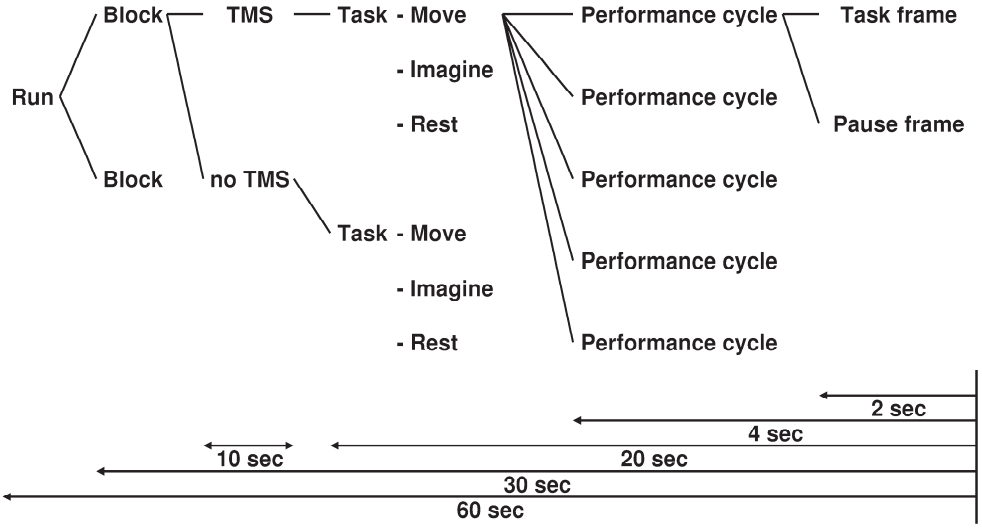

Figure 1 *- Schematic overview of the experimental set-up. The experiment contained 15 runs of 1 minute, each run contained 2 blocks of 30 seconds. Each block was started with or without 10 seconds TMS followed by a task. Each task was performed in 5 cycles containing a 2-second task frame followed by a 2-second pause frame. Three different tasks were performed: movement execution, movement imagery and rest.*

Interleaved TMS/MRI

Using interleaved TMS/fMRI in a 3 Tesla MRI scanner (Philips, Best, the Netherlands) with specially built head coil for TMS (Nova Medical Inc., Wakefield, MA, USA), BOLD sensitive single-shot echo-planar imaging fMRI images were acquired continuously (repetition time (TR) = 2300ms, field of view= 23 cm, 23 slices of 3.5 mm thickness with 64*64 matrix for 392 time points). During scanning, individual TMS pulses at 1 Hz frequency and 115% of motor threshold intensity were applied over the left superior parietal cortex [MNI stereotactic space coordinates x -24, y -60, z 68] in trains of 10 pulses. A train stopped 1 second before the task started. Motor threshold was defined as a percentage of the stimulator output that evoked a clear response in the right target muscle (thumb) in 5 out of 10 pulses on the left primary motor thumb region. TMS was applied with biphasic pulses from a Magstim Rapid® (The Magstim Company Ltd, Whitland, Wales, UK) with special non-ferromagnetic, figure-of-eight TMS coil. The coil was

designed to withstand the mechanical stresses of a high-field MRI environment. The coil was connected to an eight meter long cable and a custom filter box outside the magnet room (Bohning et al., 1998).

The synchronization of TMS and the fMRI acquisition was determined by a pulse sent out by the fMRI at the beginning of each TR period. Lab View software (National Instruments, Austin, Texas, USA) picked up these pulses and maintained a pulse count. It then triggered the TMS pulse based on an event list with the TR period and the delay in milliseconds into that TR period for insertion of the TMS pulse. The TR period was 2300 ms divided into 23 slices scanned at 100ms intervals. In order to prevent the firing of the TMS pulse during the MR acquisition radio frequency excitation pulse, the TMS pulses were always inserted 19 ms after the start of a 100 ms slice acquisition period. Each series of TMS pulses consisted of a train of 10 pulses, one each second, for a 1Hz frequency. For example, if the first TMS came at 19 ms into the first TR, the second pulse would come at 1019 ms, the third at 2019 ms, and the fourth at 3019 ms, or 3019-2300=719 ms into the TR period. Despite firing the TMS so that it missed the radio frequency excitation pulses, the after-effects of the pulse sometimes resulted in partial blackening of the slice currently being acquired. Hence, those slices were replaced with the average of the corresponding slices before and after the compromised slice. By either increasing the TR, or reducing the number of slices we could have made the slice acquisition interval long enough to insert the TMS pulse without compromising those images, but that would have made either the scan too long or would have forced us to use slices that were too thick.

To position and fixate the TMS coil inside the MRI head coil a TMS/fMRI holder was designed (Bohning et al., 2003). This device allowed the operator to manually move the TMS coil in 6 scaled degrees of freedom to a point on the subject's scalp and set its orientation so as to stimulate the selected target in the cortex. The MNI normalization space coordinates for the target [left superior parietal cortex, x -24, y -60, z 68; Figure 2] were based on results from a previous study (***chapter 2***). Before positioning of the coil, all subjects underwent a T1-weighted anatomical scan (1x1 mm^2 resolution, 1 mm slice thickness). This scan was loaded into a MR-guided positioning software package and fitted on a MNI template. The software

placed the MNI target coordinates as a virtual marker on the scalp in the anatomical images. The resulting images with the marker were refitted on the subject's anatomical scan followed by the computing of the TMS/fMRI holder settings needed to position the coil over the actual target area. This system is adapted from an earlier MR-guided positioning system that was described in a previous article from our group (Bohning et al., 2003).

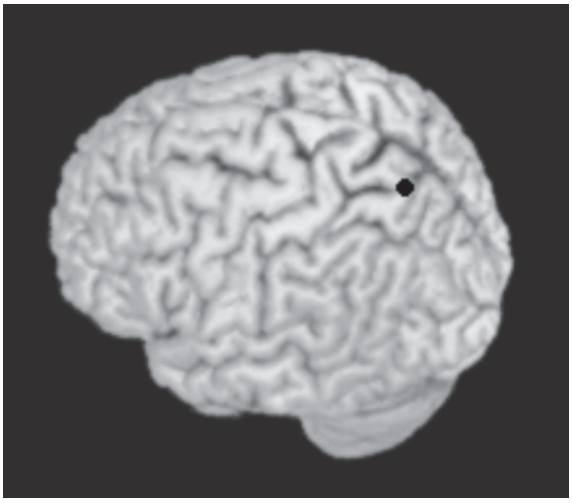

Figure 2 *- Anatomical localization of the TMS stimulation site. TMS was targeted at the left superior parietal cortex at the coordinates x -24, y -60, z 68.*

Analysis, data pre-processing

The fMRI images were first checked for any compromising slices by the TMS pulse. This resulted in an fMRI artifact induced by TMS in an average group percentage of 0,6% of all scanned slices per subject. The first two subjects had a TMS related artifact in all slices that were scanned during TMS (160 slices) which was 1,8% of the total scan slices. Subsequent adjustments to the MRI scan slice intervals were made resulting in an artifact percentage of 1,3% (2 subjects, 115 slices) and 0% (6 subjects). Identification of the slices that were compromised was done in two ways. After the discovery of partial blackening of the whole brain mask in SPM during pre-processing of the scan series, the images were realigned again. The graph of the re-realignment showed a spike at the scan slice that was

compromised. We also performed a visual check of the volume of interest time series of each beta file. A list of numbers was generated and coupled to a scan slice. We could thus identify large negative numbers which were linked to specific scan slice numbers. This also ascertained us that the slice previous and after the compromised slice was normal. The compromised slices were removed and replaced with the average of the corresponding slices before and after the compromised slice. This adjusted set of data was then loaded in SPM2 (Wellcome Dept. Cognitive Neurology, London, UK) for image realignment, transformation into standard stereotactic space (MNI template), smoothing (6x6x6 mm) and statistical analysis.

Statistical Analysis

For statistical analysis with SPM, each task (execution, imagine) was matched with the rest condition that was used as a control condition (e.g. task after TMS vs. rest after TMS). A first-level analysis with a one-sample t-test was used to assess the change in cerebral activity for each condition (execution, imagine, rest) after TMS compared to the same condition without TMS. The direct effect of TMS on the target area was measured by comparing BOLD responses associated with the 10-second time frame in which TMS was applied and a 10-second period without TMS. A second-level analysis was performed with a two-sided t-test to compare the tasks after TMS with the tasks without TMS (execution vs imagine after TMS compared to execution vs imagine without TMS). For the assessment of statistical significance the analysis included a threshold of $P<0.001$ (voxel height response), with cluster size above 8 voxels and cluster-level correction of $P<0.05$. In order to determine the extension of significant foci as well as to assess expected trends in activation changes, a relaxed threshold of $P<0.01$ voxel height response threshold ($P<0.05$ cluster corrected) was applied.

Results

None of the subjects reported any side-effects of the experiment apart from slight head discomfort due to pressure of the TMS coil.

Task ratings

The difficulty of the tasks was scored by the subjects on a 1-5 scale (impossible – normal). According these ratings (mean ± SD), task difficulty remained generally the same before scanning (execution: 4.6 ± 1.0; imagery 3.9 ± 1.4), immediately after (execution: 4.2 ± 1.0; imagery 3.8 ± 1.4) and 30 minutes after scanning (execution: 4.7 ± 0.9; imagery 4.3 ± 1.1). Only movement execution was rated significantly more difficult immediately after scanning compared to execution before and 30 minutes after scanning (P=0.037).

Group effect of tasks without TMS influence

Movement imagery (imagery without TMS vs. rest without TMS). Imagery of movement was related with activations in the dorsal premotor cortex of both hemispheres [BA6]. On the medial frontal surface, the dominant cluster of activation included the SMA and adjacent cingulate gyrus, while at a more anterior location, bilateral superior frontal gyrus activation was seen. Activations were additionally seen in the right middle frontal gyrus, right inferior parietal and right superior temporal cortex (Figure 3a, Table 1). Subcortically, right putamen activation was observed.

Movement execution (execution without TMS vs. rest without TMS). Prominent movement-related activation was found in the contralateral (left) sensorimotor cortex and adjacent dorsal premotor cortex [BA4/6]. Medial frontal activation included the left SMA [BA6], while additional activations were distributed over the right superior and middle frontal gyrus [BA8,6], bilateral superior and right inferior parietal cortex [BA7,40], right fusiform gyrus and left superior temporal cortex [BA12] [BA37] (Figure 4a; Table 1).

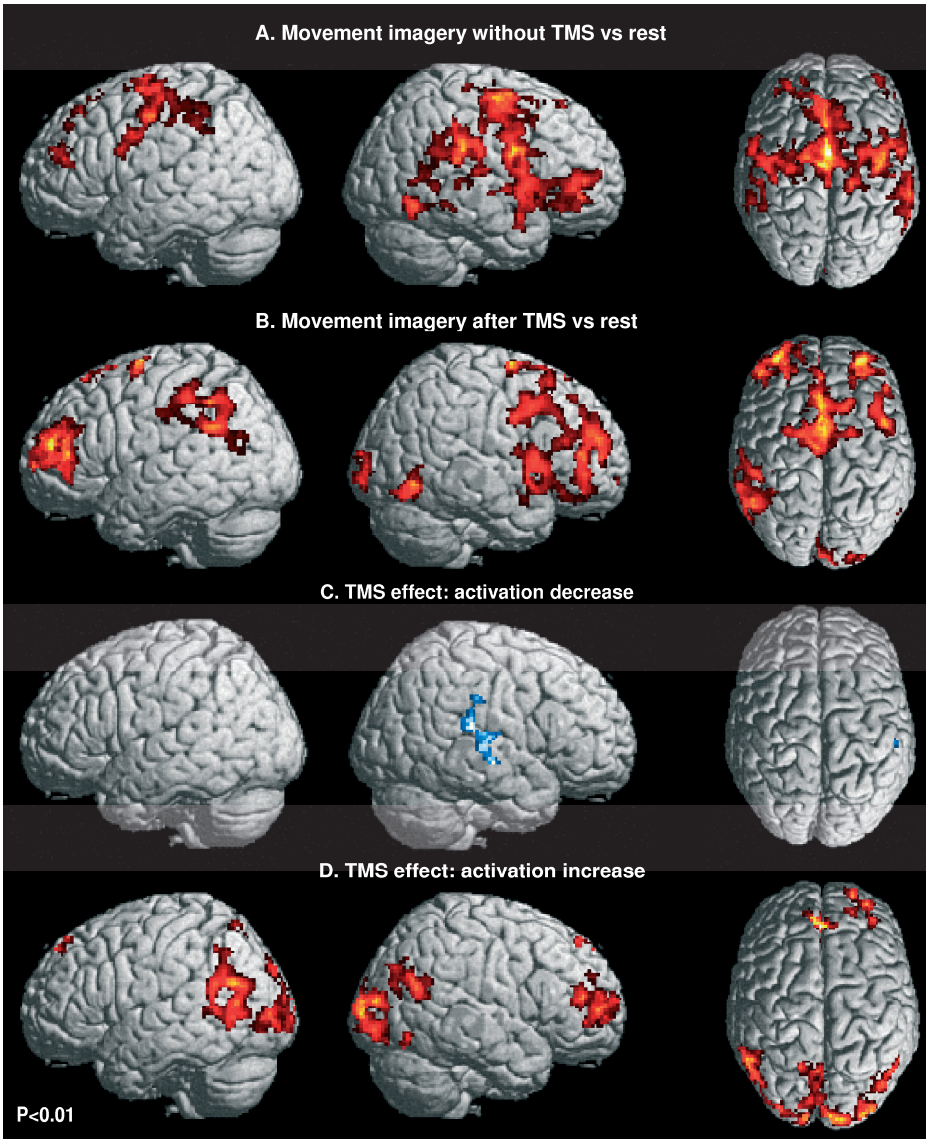

Figure 3– *Activation patterns during movement imagery task without and after TMS rendered onto a standard brain volume (contrast A – imagery without TMS vs rest without TMS; contrast B - imagery afterTMS vs rest afterTMS). The effects of TMS are shown by the decrease of activation in the right temporo-parietal cortex (contrast C – imagery without TMS vs imagery after TMS) and the increases of activation in the medial prefrontal, left supramarginal gyrus and bilateral occipital cortex (contrast D – imagery after TMS vs imagery without TMS). The initial voxel threshold was set at P<0.01, with cluster-level correction of P<0.05. Coordinates of significant clusters (P<0.001, corrected) are given in Table 2.*

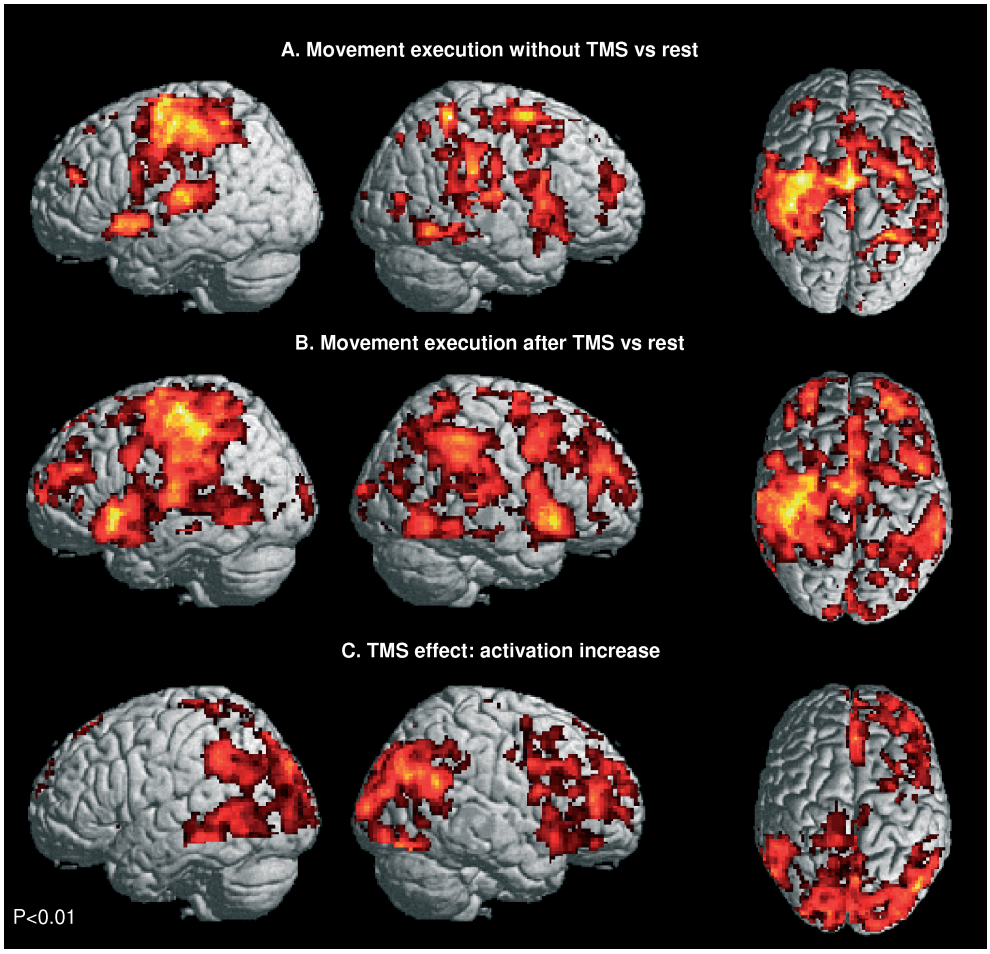

Figure 4 – *Activation patterns during the movement execution task without and after TMS rendered onto a standard brain volume (contrast A – execution without TMS vs rest without TMS; contrast B - execution after TMS vs rest after TMS). The effect of TMS can be seen in the lower row which demonstrates the increased activations in right prefrontal cortex, left inferior parietal region and bilateral temporal cortex (contrast C – execution after TMS vs execution without TMS). The initial voxel threshold was set at P<0.01, with cluster-level correction of P<0.05. Coordinates of significant clusters (P<0.001, corrected) are given in Table 2.*

Movement execution versus movement imagery (execution without TMS vs. imagery without TMS). The patterns of activation related to movement imagery and execution, without TMS, showed an overall similarity, with exception of the contralateral sensorimotor cortex, which was only activated during execution. The formal statistical comparison indeed revealed significant additional activation during movement execution in the left sensorimotor cortex, but also in the left inferior parietal cortex, left superior temporal cortex and bilateral anterior cerebellum. This statistical analysis did not show increased activations during movement imagery when compared to execution (Table 1).

Group effect of tasks after TMS application

Movement imagery (imagery after TMS vs. rest after TMS/ imagery after TMS vs imagery without TMS). Contrasting movement imagery after TMS with rest after TMS, increased activations were seen at bilateral prefrontal cortex and occipital cortex, while significant activations in the premotor and antero-ventral parietal cortex were absent (Figure 3b). A reduction of (particularly right) temporo-parietal activation due to TMS was established at voxel-level $P<0.001$ (uncorrected). After initial voxel threshold relaxation, this decrease of activation was statistically significant (cluster-corrected $P<0.05$) in the right superior temporal gyrus extending to the inferior parietal cortex [BA41; x 50, y -18 z 10; x 56, y -28, z 34] (Figure 3c).

When imagery after TMS is compared to imagery without TMS, left superior parietal TMS preceding movement imagery resulted in a significant increase of task-related activations at anterior prefrontal locations, i.e. in left medial [BA9] and right middle frontal gyrus. In addition, increased activations were seen at posterior locations in the brain, comprising the left supramarginal gyrus [BA40], as well as the right middle and inferior occipital gyri [BA18] (Table 2; Figure 3d).

Movement execution (execution after TMS vs rest after TMS/ execution after TMS vs. execution without TMS). When movement execution after TMS was compared with rest after TMS, activations in the premotor and anterior parietal cortex remained present (Figure 4b), which was different from the effect of TMS on imagery of movement (Figure 3b). Indeed, TMS did not induce significant

decreases in execution-related activation, neither at the regular voxel-response threshold, nor at the relaxed threshold (P<0.01, uncorrected).

When compared to movement execution without TMS, interleaved TMS in the right-hand execution condition resulted in an increased activation of the right medial frontal gyrus [BA8] and inferior frontal gyrus [BA45], bilaterally. In posterior parts of the brain, increased activations were seen at the right temporo-parietal junction, in the posterior cingulate gyrus, left postcentral gyrus [BA5/7], left inferior parietal cortex [BA40], middle temporal gyrus and bilateral cuneus [BA19] (Figure 4c, Table 2). Subcortically, gray matter activations were seen in the left thalamus and putamen.

Movement execution versus movement imagery (execution after TMS vs. imagery after TMS). At visual inspection, the activations after TMS were more robust during movement execution than during imagery. Formal statistical comparison indeed confirmed that the execution of movement resulted in extensive additional activation increases when compared to imagery. These significant increases were seen in the left sensorimotor cortex, cingulate gyrus, right inferior frontal gyrus, bilateral inferior parietal regions [BA40], bilateral temporal cortices, cerebellum and putamen. No significant regional increases related to imagery were seen, when compared to execution (Table 2). Finally, the comparison between the differences of the two tasks, either after TMS or without TMS, resulted in an activation increase in the right inferior parietal cortex [x 54, y -38, z 40] related to execution after TMS (P<0.01, uncorrected).

Local effects of TMS

The effect of TMS itself, without any task performance, was measured by contrasting the BOLD response evoked during the 10-second TMS application to the 10-second non-TMS frame (Figure 1). This comparison resulted in an increased neural activity in the right middle frontal gyrus [BA9], right superior and middle temporal gyrus [BA22] and left superior occipital cortex [BA19] (Table 2). Left temporal gyrus activation [x -56, y -24, z 4] was also present at the same P-value of 0.001, but only significant at cluster-correction of P=0.06 (T=5.95; Z=3.7).

These activations logically included the effect of discharge noise of the TMS coil. A comparison between the rest conditions that followed either the TMS frame or the non-TMS frame did not result in significant activation increases. This comparison enabled us to assess a possible prolonged effect of resting state TMS at a time frame similar to that of the performed movement tasks.

Discussion

The present results demonstrated changes in the distribution of task-related cerebral activations in HC, immediately after 10 pulses of 1 Hz TMS over the left superior parietal cortex. During both movement execution and movement imagery, of which the latter is regarded to represent an equivalent of movement preparation (Ehrsson et al., 2003; Gieteling et al., 2008), TMS was related with an increase of activations in bilateral prefrontal and posterior parietal cortices, when compared to the tasks without TMS. The distribution of these increases showed a general resemblance between the two task conditions. No regional decreases were seen after TMS in the movement execution task. In movement imagery, however, particularly antero-ventral parietal activation was reduced after TMS. In other words, priming TMS coincided with (i) increased activation occurring in regions that have only indirect connections with the M1, while (ii) decreased activation did occur within circuitry directly implicated in higher-order motor control (Georgopoulos, 1991; Rizzolatti and Luppino, 2001).

TMS effects during resting state

The effects of TMS in resting state, unrelated to task performance, were assessed in two ways. The direct effect of TMS was studied by comparing the 10-second stimulation frame with the 10-second non-TMS frame. The possibility of a prolonged TMS effect in rest was examined by contrasting the rest condition after TMS to the rest condition without TMS. The direct TMS effect included activation of the auditory cortex, which is a logical consequence of the noise of TMS itself. The increased activity in the right middle frontal gyrus during TMS cannot be simply attributed to auditory stimulation. One might speculate that it reflected an aspect of attention or evaluation in the context of the previously given task instructions

(Baudewig et al., 2001). These activations did not last beyond the 10-second TMS frame, as shown by the absence of increased activation in the comparison of rest after TMS with the equivalent rest without preceding TMS. No changes of activation were seen at the left superior parietal target site itself. Although some studies have shown activation changes at the target area (Bestmann et al., 2003; Bohning et al., 1998), other interleaved TMS/ fMRI studies did not (Baudewig et al., 2001; Bohning et al., 1999; Kemna and Gembris, 2003). Several explanations have been proposed for the lack of activation changes at the target area. It has been noticed that TMS induces a signal reduction under the coil that may reduce the statistical significance of activation in this target area (Bohning et al., 1999). Possibly, such signal reduction represents a still unclear neuronal inhibitory effect of TMS (Bohning et al., 1999) or modulation of the input from afferent projections to the cortex (Baudewig et al., 2001). In this respect, we may have to consider the possibility that indeed the sum effect of TMS is not primarily expressed in a local perfusion change (Baudewig et al., 2001).

The fact that a local TMS effect may or may not occur, might even be region-specific. In resting state, interleaved suprathreshold 4Hz TMS on the posterior parietal cortex has been reported not to induce a change in BOLD activation, while it did on the primary sensorimotor cortex (Kemna and Gembris, 2003). The latter, however, was explained by the sensory consequences of the elicited motor reaction. Similar to that study, parietal TMS during resting state in our experiment did not evoke activation changes in wider distributed circuitry either. This provides an argument that the pattern of TMS-linked changes of task-related activations is not explained by an aspecific effect of TMS itself. An even stronger argument that the task-related changes were not aspecific TMS effects is obtained by the fact that no significant activation changes were seen in rest after TMS. This allowed for a direct comparison between the task conditions after and without TMS.

Task-related activation changes after TMS

The strongest effects associated with TMS were the activation increases outside the target area. Both during execution and imagery of movement, TMS was followed by a similar pattern of increased activation in bilateral prefrontal and

posterior parietal cortices. Although the anterior parietal decrease of activation after TMS was subtle, its distinct location and interconnection (Fang et al., 2005; Johnson et al., 1996) provide legitimate arguments for our inference that the remote increases of activation reflect a compensatory mechanism. These increases were at more distant locations from M1 than parietal-premotor circuitry which is closely centered around M1. This parietal-premotor circuitry makes crucial contributions to sensorimotor transformations that are required for adequate movement (Rizzolatti and Luppino, 2001; Wise et al., 1997). Within this circuitry, a general gradient of increasing movement-related responses can be discerned towards M1, both in posterior direction within the premotor cortex and in anterior direction in the parietal cortex (Johnson et al., 1996). This fits the description that simple movement execution is coordinated in the highly specialized movement-related areas in and directly bordering M1, while more complex movement tasks require additional input from less specialized secondary areas, including ventral premotor and anterior parietal cortices, to achieve an adequate task accomplishment (Rizzolatti and Luppino, 2001). Therefore, if the input from adjacent (secondary) regions to M1 is still insufficient, robust activation of the least-specialized (tertiary) areas such as prefrontal and posterior parietal regions are recruited. In this respect, the medial prefrontal and left posterior parietal cortex are well-suited to act at the upper hierarchical levels in the cerebral organization of movement, that is characterized by an increasing specificity of prepared movements towards the final cortical output channels in M1 (de Jong et al., 1999; de Jong and Paans, 2007; Rushworth et al., 2003).

This principle of hierarchical organization has also been demonstrated with TMS. Applying a virtual lesion in the primary motor region showed compensation in the dorsal premotor region (Lee et al., 2003), while a TMS lesion in the dorsal premotor region led to compensation in the cingulate motor area and SMA (O'Shea et al., 2007). When TMS manipulation is targeted at remote locations from the motor cortex, such as the dorsolateral prefrontal region, the brain can still compensate the lesion with robust activation increases in the prefrontal and parietal cortices respectively (Rounis et al., 2006). This compensatory potency has not only been functionally identified, but has also been anatomically described in neurological

conditions (Calautti and Baron, 2003; Maruishi et al., 2007; Staffen et al., 2002). The referred studies thus converge in the notion that compensation within a disturbed functionally coherent network particularly challenges the least-specialized nodes within the network in order to redirect the preparation of a given task.

Sensorimotor integration

The increased activation in the posterior and superior parietal cortex occurred during both imagery and execution, while the antero-ventral parietal activation after TMS was only present in movement imagery. This raises the question why the 'compensatory' posterior parietal increases after TMS were not associated with anterior parietal decreases during movement execution. In this respect, the effect of sensory feedback during movement execution needs to be considered.

The antero-ventral parietal activation decrease during movement imagery points at changes in the representation of information based on higher-order proprioception. This portion of the parietal lobe, along the Sylvian fissure, contains cortical fields such as the S2 and parietal ventral area that have been implicated as an interface between proprioceptive information processing and the organization underlying motor control (Hinkley et al., 2007). Even without external sensory activation, such somatosensory circuitry contributes to movement preparation by means of corollary discharge (Christensen et al., 2007; McCloskey, 1981). The latter implies that movement planning includes anticipation on the proprioceptive consequences of movement. As we consider movement imagery to some extent as an equivalent of movement preparation (Ehrsson et al., 2003), the TMS-induced decrease of antero-ventral parietal activation would thus indicate an affected ability of integrating the somatosensory consequences of movement during the preparation of such movement. During movement execution, on the other hand, actual proprioceptive information is obtained from the limbs. Apparently, this direct sensory input in the antero-ventral parietal cortex overrules a TMS-induced decrease of activation related to a 'preparatory motor stage', while at the same time this input is a confounding factor in the assessment of such preparation preceding the execution of overt movements. Indeed, the only effect of TMS associated with the difference between movement execution and imagery was an

execution-related increase of right inferior parietal activation. This relative 'overshoot' underscores the TMS-induced reduction of anterior parietal activation during imagery. One may therefore speculate that the shift from anterior parietal to posterior parietal activation represents a shift from a proprioceptive to a vision-based preparation strategy.

TMS methodology

The changes in activation which we identified could only be detected with the interleaved TMS/MRI technique. Although this technique has major conceptual advantages, there are also some drawbacks that need to be considered. First, 0,6% of the BOLD fMRI images in the group had an artifact induced by TMS. Artifacts can occur from static magnetic field inhomogeneities in the vicinity of TMS coils. Even the use of MRI-compatible materials could be insufficient because fMRI recordings are based on strongly T2* weighted images. Perpendicular instead of parallel TMS orientation can contribute to artifacts as well. However, Baudewig et al. speculated that these artifactual signal losses and geometric distortions do not have to occur with good equipment and a correct set-up (Baudewig et al., 2000). Therefore, the artifacts possibly appear as a result of a very small time frame for TMS: in 0,6% of the slices the period in which the after-effects of the TMS pulse were present, may have been too close to the start of the MRI slice acquisition. Second, the positioning of the TMS coil is an important confounder. In contrast to primary motor cortex targeting, no visible feedback of the coil position on the left superior parietal cortex can be acquired. We tried to minimize this confounder by using the newest MRI-guided positioning system based on the subject's own anatomical scan. Although this system is not optimal, a previous study has shown that this system can place the TMS coil more accurately than the functional method of manual localization (Bohning et al., 2003).

Conclusion

Applied in a simple right-hand movement paradigm, TMS related changes of activation of the left superior parietal cortex leads to specific changes in task-related activation, distributed over remote parietal and prefrontal cortical areas. We

found increased activation of posterior parietal cortex and prefrontal cortex during both movement execution and imagery. In addition, a decrease was seen in the anterior parietal region during movement imagery after TMS. The combination of these changes indicates that disturbance as well as compensation occurred within circuitry that enables the integration of somatosensory information for effective motor control.

Appendix for chapter 4

Table 1. Activation patterns during task without TMS influence

Brain region (Brodmann areas)		Left			Z	Right			Z
		x	y	z	score	x	y	z	score
Movement imagery: Increased activation									
Frontal lobe	Superior gyrus	-10	30	44	3.94	12	18	56	4.04
	Anterior cingulate gyrus	-2	14	24	5.22				
	SMA (BA6)					0	-6	58	4.66
	Middle gyrus (BA46)					40	46	6	5.27
	Precentral gyrus (BA6)	-48	-12	56	3.74	42	-12	50	4.34
						26	-12	60	3.69
						56	0	24	5.31
Parietal lobe	Inferior gyrus (BA40)					64	-32	26	4.51
Temporal lobe	Superior gyrus					50	8	2	3.99
Subcortical	Putamen					24	2	-2	3.99
Movement execution: Increased activation									
Frontal lobe	Superior gyrus (BA8)					10	18	54	3.95
	Middle gyrus (BA6)					28	2	56	4.26
	SMA (BA6)	-10	-10	70	4.18				
	Inferior gyrus (BA6) (premotor ctx)					52	8	10	3.74
	Precentral gyrus (BA4/6) (primary motor with dorsal premotor ctx)	-40	-22	64	4.32				
Parietal lobe	Postcentral gyrus (BA40)	-54	-18	14	4.16				
	Superior gyrus (BA7)					30	-48	60	4.75
	Inferior gyrus (BA40)					46	-34	38	3.53
Temporal lobe	Fusiform gyrus (BA37)					52	-64	-12	3.84
	Superior gyrus (BA12)	-58	10	-8	3.93				
Movement execution vs movement imagery: Increased activation during execution									
Parietal lobe	Postcentral gyrus (sensorimotor ctx)	-44	-30	48	4.90				
	Inferior gyrus (BA40)	-38	-48	58	4.20				
Temporal lobe	Superior gyrus (BA41)	-52	-18	14	3.77				
Cerebellum	Anterior	-4	-54	-18	4.80	2	-64	-16	3.47

Localization of significant task-related activations by SPM [Z], not influenced by TMS. The regional activations resulted from comparisons of movement execution vs rest, movement imagery vs rest and movement execution vs movement imagery, respectively. Positive x-y-z coordinates (in mm) indicate locations respectively right, anterior and superior to the middle of the anterior commissure. Initial threshold for response height at voxel-level was P<0.001 with cluster-level correction for the entire brain at P<0.05. Degrees of freedom are [1.0, 9.0].

Table 2. Effects of parietal TMS on cerebral activation

Brain region (Brodmann areas)		Left			Z	Right			Z
		x	y	z	score	x	y	z	score
Direct TMS effect without a task: Increased activation									
Frontal lobe	Middle gyrus (BA9)					44	16	30	4.63
Temporal lobe	Superior gyrus (BA22)					52	-10	-2	4.66
	Middle gyrus					60	-34	0	4.64
Occipital lobe	Superior gyrus (BA19)	-34	-72	30	3.81				
Movement imagery: Increased activation									
Frontal lobe	Medial gyrus (BA9)	-6	38	32	5.21				
	Middle gyrus (anterior)					30	56	8	3.94
						36	44	14	3.79
Temporal lobe	Supramarginal gyrus	-56	-54	24	4.44				
Occipital lobe	Middle gyrus					28	-90	12	4.18
	Inferior gyrus (BA18)					14	100	-4	3.77
Movement execution: Increased activation									
Frontal lobe	Medial gyrus (BA8)					4	32	42	3.66
	Middle gyrus (BA46)					34	38	14	4.03
	Inferior gyrus (BA45)	-42	28	16	3.85	48	16	-10	3.92
Temporal lobe	Temporo-parietal junction					48	-72	28	5.41
	Middle gyrus	-60	-42	-10	3.94				
		-54	-68	28	4.03				
Parietal lobe	Postcentral gyrus (BA5/7)	-16	-42	66	3.8				
	Posterior cingulate gyrus					4	-44	26	4.23
	Inferior gyrus (BA40)	-52	-44	38	4.09				
Occipital	Cuneus (BA18)	-16	-92	32	4.59	26	-100	10	3.73
Subcortical	Thalamus	-16	-20	0	3.87				
	Putamen	-22	12	2	4.16				
Cerebellum	Posterior					30	-70	-16	4.03
Movement execution vs movement imagery: Increased activation during execution									
Frontal lobe	Inferior gyrus					44	4	6	3.86
	Cingulate gyrus	-4	-8	42	3.96	4	26	20	4.22
Parietal lobe	Postcentral gyrus	-64	-18	26	3.84				
	Inferior gyrus (BA40)	-42	-30	46	4.87	52	-38	42	4.40
Temporal lobe	Superior gyrus (BA22)	-52	-16	4	3.82	42	-28	14	4.10
	Inferior gyrus (BA37)					54	-60	-18	3.97
Cerebellum	Anterior	-4	-54	-18	3.48	6	-62	-18	3.31
Subcortical	Putamen	-28	-10	12	4.07	20	10	12	4.35

Localization of the increases in task-related activations caused by TMS, identified by SPM [Z]. In addition to the task-related effects, the direct TMS effect was assessed by contrasting TMS frame to the equivalent frame non-TMS (see also Figure 1).

Chapter 5

Prefrontal and parietal adaptations in cervical dystonia after parietal TMS interleaved with fMRI

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In preparation

Abstract

Clinically normal hand movement associated with altered cerebral activation patterns in CD may imply cerebral adaptation. Since impaired sensorimotor integration appears to play a role in dystonia, left superior parietal cortex modulation with TMS was employed to further challenge adaptation mechanisms reflected by a change in cerebral activation. Seven CD patients and ten HC were scanned on a 3T MRI scanner with interleaved TMS. They executed and imagined right wrist flexion/ extension movements. Each task was preceded by a 10-second period either with or without TMS. TMS in HC showed a similar task-related activation pattern as found in CD without TMS influence, i.e. increases of activation in bilateral prefrontal and posterior parietal regions during both tasks and decreases in right anterior parietal cortex during movement imagery ($P<0.001$). The TMS effect in CD was weaker but with a similar trend in activation changes. Only in the right angular gyrus, TMS significantly failed to induce an increase of activation in CD as was seen in the control subjects ($P<0.001$). The similarity between TMS effects on the distribution of cerebral activations in controls and the pattern seen in CD supports the concept that CD patients make use of compensatory circuitry enabling clinically normal hand movement. The fact that a similar but weaker TMS effect occurred in CD suggests that the capacity of compensation is reduced. Particularly for the right angular gyrus, this reduction was statistically significant.

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Introduction

CD is defined as a movement disorder with abnormal involuntary muscle contractions and postures of the head and neck. The execution of hand movement is clinically normal. However, we have recently demonstrated with fMRI that the cerebral organization underlying hand movements in CD differs from normal (***chapter 2***), while subtle changes in muscle contraction were also found by EMG (***chapter 3***). The association of apparent normal hand function and a deviant distribution of cerebral activation suggests a flexibility of the brain to adapt to impaired cortical function by recruiting other cortical areas to perform the desired task. This issue was further addressed in the present study.

The cause of CD is unknown. On the other hand, neuroimaging studies have reported abnormal function of brain areas during task performance in a dystonic body part. This method has demonstrated overactivation in the basal ganglia and prefrontal cortex (Ceballos-Baumann and Brooks, 1997; Playford et al., 1998). Abnormal activity was also seen in S1/M1 and adjacent sensorimotor-related areas, which were either underactivated (Blood et al., 2004; Gieteling et al., 2008) or overactivated (Odergren et al., 1998; Pujol et al., 2000). Several studies have tried to define a possible explanation for this impaired function of the sensorimotor cortex (Abbruzzese and Berardelli, 2003) (***chapter 2***). The parietal cortex plays an important role in the higher-order sensory processing. It contains information of (visuo)spatial perception, body scheme and proprioception in order to integrate this in the preparation of a (spatial) movement (de Jong et al., 2001; Rushworth et al., 2003). Changes in activation may reflect a deficit in the processing and integration of this sensory information. Modulation of this higher-order sensory processing can be recognized in overt or imagined sensory tricks that may alleviate dystonic symptoms (Naumann et al., 2000; Schramm et al., 2004) or sensory signals that have been reported to precede the onset of dystonic symptoms. Interestingly, voxel-based morphometric structural changes have also been reported for the sensorimotor area in CD (Obermann et al., 2007).

The widespread changes in the cerebral networks in CD may seem at odds with the fact that dystonic symptoms are usually only localized in one body part, particularly in the neck. Movement in other body parts than the neck is clinically normal. However, investigation of this discrepancy in CD with fMRI during movement of a non-dystonic body part resulted in similar abnormal cerebral activations as has been reported for dystonic body part movement (***chapter 2***). It was hypothesized that this association between reduced activation in movement associated brain regions and clinically normal movement performance may imply an effective compensation mechanism in CD. The brain's ability to adapt to new situations has been seen in clinical neurological conditions as stroke (Calautti and Baron, 2003) or multiple sclerosis (Staffen et al., 2002). In an experimental setting, TMS induced virtual lesions in primary motor cortex (Lee et al., 2003), dorsal premotor regions (O'Shea et al., 2007) and dorsolateral prefrontal cortex (Rounis et al., 2006) also showed compensatory activation changes. Compensation also occurred after TMS induced modulation of the superior parietal cortex in HC; 'compensatory' increases were seen in posterior parietal and prefrontal networks during hand movement execution and imagery (***chapter 4***).

As the patterns of changed activation were observed in CD as well as in HC after TMS, a mechanism of compensation can be suggested. For this study, we aimed to investigate to what extent such compensation can be further challenged by TMS in CD. We wanted to assess the adaptation of activation necessary to still be able to perform clinically normal movement in a non-dystonic body part. We therefore introduced movement execution and imagery tasks. Movement imagery is described to reflect movement preparation, with the recruitment of movement programs to simulate movement performances without executing overt movement. It recruits the same neural networks in the motor system that are engaged when movement is actually being executed (Ehrsson et al., 2003). In order to challenge possible compensatory networks, we chose to induce virtual TMS lesions on the superior parietal cortex. Effects of changes in activation during the movement tasks were measured directly with fMRI (interleaved TMS/fMRI; (Bohning et al., 1998)). The choice of the superior parietal cortex is based on fMRI studies which, in most cases, clearly show underactivation in this specific area (Blood et al., 2004)

(***chapter 2***). Further inhibition of this region with TMS might stress the compensatory networks even more. These compensatory networks are assumed to include (i) the prefrontal cortex, since it is already overactivated in CD, presumably as an adaptation to impaired parietal function and (ii) posterior parietal regions, clearly showing adaptive increases in HC after superior parietal cortex modulation.

Methods

Subjects

Ten HC (mean age 53 +/- 11 (SD); 8 females) and seven patients with CD (mean age 57 +/- 16 (SD), 6 females) were studied. Six patients had CD (one with concurrent spasmodic dysphonia), and one patient had generalized dystonia (no DYT1 mutation carrier). All patients presented with CD as their predominant clinical symptom (Table 1). The subjects signed a written informed consent approved by the Medical University of South Carolina institutional review board. All were right-handed, assessed by the Annett Handedness Scale (Annett, 1970). No subject had a medical history of seizures or neurological disorders except primary dystonia. Each subject underwent one 15-minute session during which the effect of interleaved TMS/fMRI was assessed.

Behavioral task

This study used movement execution and imagery tasks. Although movement imagery has been used before in neuroimaging studies (Hanakawa et al., 2003), it is hard to monitor if and how subjects perform the task inside an MRI scanner. A few weeks prior to the experiment, we recruited subjects who had an average image performance based on the Vividness of Movement Imagery Questionnaire (Isaac et al., 1986) and the speed of performance task (***chapter 4***). Although CD had a higher score on the questionnaire than controls, this difference was not significant (Table 2). In the speed of performance task, two cycles of 10 movement executions and 10 imagery movements were timed. The speed of performance test

showed that it took CD significantly longer to imagine 10 cycles of hand movement than controls (P=0.013; Table 2).

During the fMRI scan, subjects performed right-hand tasks in executive, imagery or rest mode. See ***chapter 4*** for details on these tasks and the fMRI paradigm. The hand was held in an armrest for support while the fingers were holding a vertical handle mounted to a short arm that pivoted just under the wrist. The extent of the movement execution was registered by recording the movement of the handle about the pivot. This registration was also used to check if subjects indeed refrained from movement during the imagery task and rest period. The sensor in the arm rest showed that all subjects performed the execution task correctly. No overt hand movements were observed during the imagery or rest tasks. Before the experiment started, subjects practiced the tasks for 5 minutes. During this practice, immediately after scanning (to evaluate the tasks during scanning) and 30 minutes after scanning, subjects scored the difficulty of the tasks on a 1-5 scale (impossible-normal). The ratings revealed for both groups that movement execution during scanning was significantly harder than during practice (P=0.037; Table 2).

Interleaved TMS/MRI

Using interleaved TMS/fMRI in a 3T MRI scanner (Philips, Best, the Netherlands) with specially built head coil for TMS (Nova Medical Inc., Wakefield, MA, USA), BOLD sensitive single-shot echo-planar imaging (EPI)-fMRI images were acquired continuously (TR = 2300ms, FOV= 23 cm, 23 slices of 3.5 mm thickness with 64*64 matrix for 392 time points). During the scanning, individual TMS pulses at 1 Hz frequency and 115% of motor threshold intensity were applied over the left superior parietal cortex [MNI stereotactic space coordinates x -24, y -60, z 68] in trains of 10 pulses. A train stopped 1 second before the task started. The stimulation was performed using a Magstim Rapid® (The Magstim Company Ltd, Whitland, Wales, UK) with special non-ferromagnetic, figure-of-eight TMS coil. The coil was designed to withstand the mechanical stresses of a high-field MRI environment. The coil was connected to an eight meter long cable and a custom filter box outside the magnet room (Bohning et al., 1998).

The synchronization of TMS and the fMRI acquisition was determined by a pulse sent out by the fMRI at the beginning of each TR period. Lab View software (National Instruments, Austin, Texas, USA) picked up these pulses and maintained a pulse count. It then triggered the TMS pulse based on an event list with the TR period and the delay in milliseconds into that TR period for insertion of the TMS pulse. See for details about the TMS-fMRI synchronization ***chapter 4***. To position and fixate the TMS coil inside the MRI head coil a TMS/fMRI holder was designed (Bohning et al., 2003). This device allowed the operator to manually move the TMS coil in 6 scaled degrees of freedom to a point on the subject's scalp and set its orientation so as to stimulate the selected target in the cortex. The MNI normalization space coordinates for the target [left superior parietal cortex, x -24, y -60, z 68] were based on results from a previous study (***chapter 2***). More details about the TMS positiong can be found in ***chapter 4***.

Analysis

The fMRI images were first checked for any compromising slices by the TMS pulse. This resulted in an fMRI artifact induced by TMS in an average group percentage of 0,6% of all scanned slices per subject. Four HC and five CD patients had compromised slices. After adjustment of the MR scan slice intervals, the artifact percentage became 0%. Identification of the compromised slices was done in two ways: re-realignment of the images and visual inspection (***chapter 4***). The adjusted set of data was then loaded in SPM2 (Wellcome Dept. Cognitive Neurology, London) for image realignment, transformation into standard stereotactic space (MNI template), smoothing (6x6x6 mm) and statistical analysis. For each task (execution, imagine) the matched rest condition was distracted (e.g. execution after TMS minus rest after TMS). Then a one-sided t-test (first level analysis) was run to investigate the change in cerebral activity for each condition (execution, imagine, rest) after TMS compared to the same condition without TMS. The resulting contrasts were compared between both groups using a two-sided t-test (second level analysis). Contrasts were significant at $P<0.001$ with cluster size above 8 voxels and cluster-level correction of $P<0.05$. If no clear significant activations were found, the threshold was lowered to $P<0.01$ (same voxel size and

cluster-level correction) in order to determine the patterns of activation and to make a comparison between groups possible.

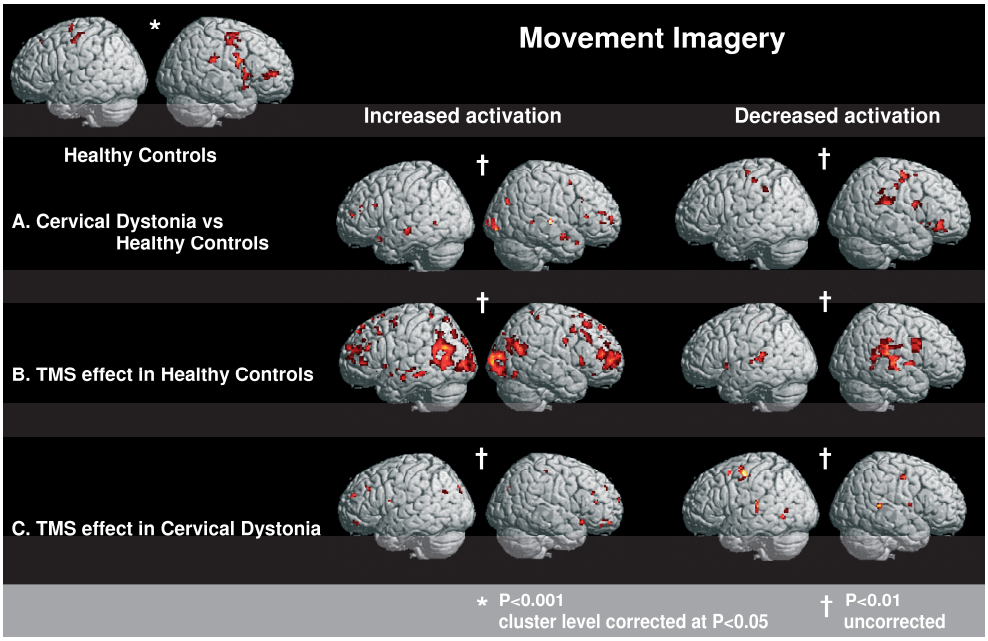

Figure 1 *– Activation patterns in cervical dystonia and healthy controls during the movement imagery task. Two thresholds are used to optimally demonstrate the characteristic global (changes in) activation patterns. In the left upper corner is the normal activation pattern depicted during imagery in controls.*

Row A *shows increased and decreased activation in dystonia compared to controls during movement imagery without TMS versus rest without TMS. Abnormal activations in dystonia are characterized by increased activity in bilateral prefrontal and posterior parietal cortices together with decreased bilateral premotor and anterior parietal regions.*

Row B *shows that left superior parietal TMS can induce a dystonic activation pattern in controls, with clear bilateral prefrontal and posterior parietal increases and a right premotor-anterior parietal decrease (contrast: movement imagery after TMS vs movement imagery without TMS).*

Row C *signifies that modulation after TMS in left superior parietal cortex in dystonia further intensifies this bilateral prefrontal-posterior parietal increases and shows decreases in left premotor and right superior temporal/ anterior parietal (contrast: movement imagery after TMS vs movement imagery without TMS)*

Results

None of the subjects reported side-effects of the experiment apart from slight head discomfort due to the pressure of the TMS coil. Dystonia patients did not notice any changes in their dystonic symptoms.

Task performance without TMS

Movement imagery (vs rest without TMS). Activations in HC can be found in ***chapter 4*** and Figure 1 left upper corner. The distribution of imagery-related activations in CD was dominated by right prefrontal and left cingulate gyrus activation. Right-sided cerebellum and left-sided thalamus activation was present in the patient group but not significantly activated in HC (Table 3). Formal comparison between the two groups with regard to movement imagery did not reveal significant activation differences. Subthreshold decreased activations in CD were distributed over the sensorimotor cortex, extending in the premotor and inferior parietal cortex (Figure 1A). Subthreshold increases of activation in CD included antero-lateral prefrontal cortices and right posterior parietal cortex ($P<0.01$) (Figure 1A).

Movement execution (vs rest without TMS). See for activations in HC ***chapter 4*** and Figure 2 left upper corner. In CD, a similar pattern of activations was seen but with more prominent bilateral prefrontal activations (Table 3). The comparisons between groups confirmed this bilateral prefrontal dominance in CD at lower threshold ($P<0.01$, Figure 2A). Lower threshold decreased activation in CD was seen in the right anterior parietal cortex (Figure 2A).

Task performance with TMS

Movement imagery In HC, the comparison of imagery after TMS versus imagery without TMS can be found in ***chapter 4*** and Figure 1B. The same comparison in CD patients showed TMS in the imagery condition evoked increased activation in the left medial frontal gyrus, only at relaxed threshold. In addition, a subthreshold trend of bilateral prefrontal increases was seen in CD, similar to the TMS effect in HC (Figure 1C). On subthreshold level, TMS-induced decreased activations were seen in the dorsal premotor cortex, particularly of the left

hemisphere (Figure 1C). A formal comparison of the TMS effect on movement imagery did not reveal significant differences in activation between the two groups.

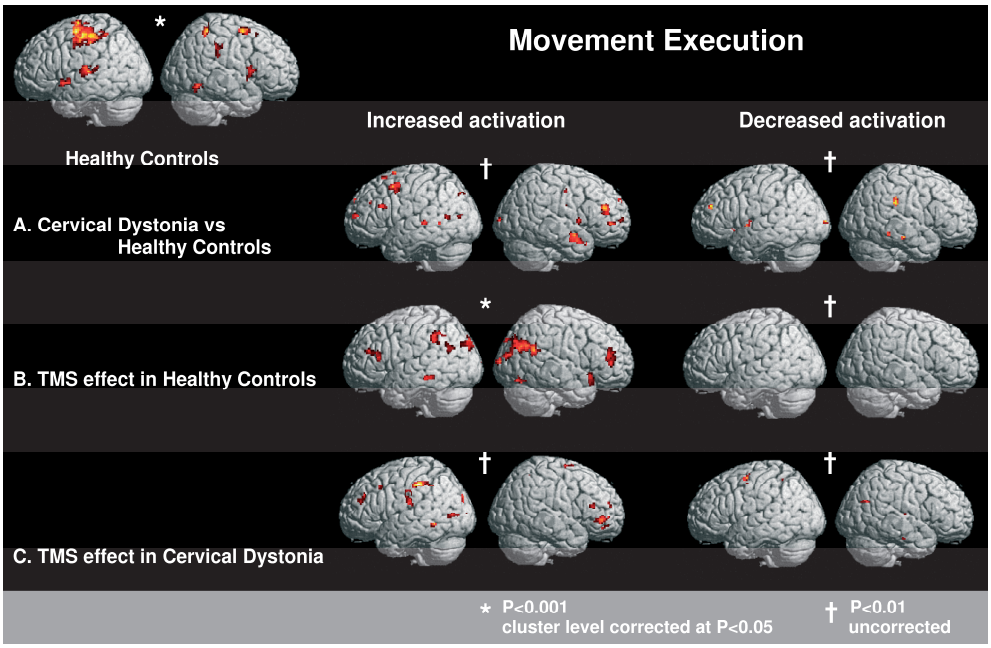

Figure 2 - *Activation patterns in cervical dystonia and healthy controls during the movement execution task. Two thresholds are used to optimally demonstrate the characteristic global (changes in) activation patterns. In the left upper corner is the normal activation pattern depicted during execution in controls.*

Row A *shows increased and decreased activation in dystonia compared to controls during movement execution without TMS versus rest without TMS. Abnormal activations in dystonia are characterized by increased activity in bilateral prefrontal and parietal cortices together with a decrease in right anterior parietal cortex.*

Row B *shows that left superior parietal TMS can induce a dystonic activation pattern in controls, with bilateral prefrontal and posterior parietal increases (contrast: movement execution after TMS vs movement execution without TMS).*

Row C *demonstrates that left superior parietal TMS further induces bilateral prefrontal increases in activation in dystonia. More posterior, decreases in left premotor and right middle temporal/ posterior parietal region and an increase in anterior parietal cortex can be observed. These results are the opposite effects as seen in movement imagery (contrast: movement execution after TMS vs movement execution without TMS).*

Movement execution When compared to the execution of movement without TMS, execution after TMS in HC showed significantly enhanced activations (***chapter 4*** and Figure 2B). CD patients had a significant activation increase only in the left inferior parietal cortex [x -46, y -30, z 46; Z 4.03]. On a relaxed threshold, activated areas were also found in right prefrontal cortex and precuneus (Figure 2C). The between-group comparison of execution after TMS versus execution without TMS showed prominent decreased activation in the right angular gyrus in CD [x 48, y -72, z 28; Z 5.66]. In other words, the only statistically significant difference between the TMS effects in the two groups was a reduced right angular gyrus activation in CD during execution.

Discussion

Aim of this study was to investigate whether the patterns of increased activation seen in CD during clinically normal hand movement are the result of flexible changes in cortical activation based on compensation mechanisms for the observed activation decreases. The abnormal cortical activations in CD were increases in bilateral prefrontal regions and decreases in bilateral anterior parietal regions and SMA (***chapter 2***). Recently, our group has found similar decreases in dystonia in patients with complex regional pain syndrome (Gieteling et al., 2008). Moreover, we were able to induce this aberrant activation pattern in HC by modulating the left superior parietal cortex with TMS (***chapter 4***). Significant increases were seen in bilateral prefrontal cortex and posterior parietal regions while decreased activation was found in the anterior parietal area during the same tasks. The similarities between the changes in activation in CD and HC after TMS provide support for our hypothesis of a compensation mechanism. Especially the prefrontal and posterior parietal cortices appear to be consistent elements of this adaptation mechanism. To what extent such compensation can be challenged, was addressed in this study. Virtual lesioning of the parietal cortex with TMS in CD showed a trend partly similar to activation changes in HC. Bilateral prefrontal cortex activations were present, but bilateral posterior parietal cortex activations were not apparent. When comparing CD with HC, the right angular gyrus in the parietal region was significantly decreased in the patient group during execution after TMS.

So, TMS in CD does induce a further challenge on the flexibility of cerebral networks but the resulting changes in activation are clearly less than in controls.

Cerebral compensation mechanisms thus seem to result in abnormal patterns of increased activation in CD. These abnormal activation patterns may be the result of an impaired function of the anterior parietal cortex in CD. The brain tries to compensate this by extra recruitment of other regions to still perform the requested movement task. The strategy of recruitment depends on the vast connections between these areas and the anterior parietal cortex that are organized according to a hierarchical scheme within motor control networks (Fang et al., 2005; Johnson et al., 1996). Simple movement execution tasks are coordinated in highly specialized movement related areas in and directly bordering the primary motor area. More complex movement tasks require additional input from less specialized secondary areas as ventral premotor and anterior parietal cortices to achieve an adequate task accomplishment. If this input is still insufficient, robust activation of tertiary areas as prefrontal and posterior parietal regions are recruited (Rizzolatti and Luppino, 2001). This principle of hierarchical organization has also been demonstrated with TMS. Applying a virtual lesion in the primary motor region shows compensation in the dorsal premotor region (Lee et al., 2003), while a TMS lesion in the dorsal premotor region leads to compensation in the cingulate motor area and SMA (O'Shea et al., 2007). When TMS manipulation is targeted at remote locations from the motor cortex, such as the dorsolateral prefrontal region, the brain can still compensate the lesion with robust activation increases in the prefrontal and parietal cortices respectively (Rounis et al., 2006).

Comparison between groups during movement execution after TMS resulted in a significant difference in activation in the right angular gyrus. In CD, this region did not present an adaptive activation increase after TMS. The right angular gyrus has been associated with movement awareness (Farrer et al., 2008). For each voluntary movement, the parietal cortex contains an internal representation of the desired movement to consciously monitor the performed movement. Discrepancies between this internal model and the actual consequences of a movement are reflected by angular gyrus activation (Farrer et al., 2008). Patients with a lesion in the right angular gyrus are able to report when they started to move, but not when

they first became aware of their intention to move (Sirigu et al., 2004). It is speculated that the lesion affects neural processes to generate mental representations of movement, another possibility may be found in an impaired feedback loop from either the moving body part or other cortical areas as the prefrontal cortex (Leiguarda and Marsden, 2000). When we extrapolate this to our results, impairments in adaptive activation increases in CD after TMS might reflect an impaired function of right angular gyrus. However, abnormal activation was not found in this region during execution and imagery of right hand movement without TMS modulation in CD (***chapter 2***). It thus seems more reasonable that impaired feedback networks are altering the adaptation process. Distortion of the cortical feedback loops may impair information processing, the "message" for additional recruitment of the angular gyrus may not have been delivered. Also, flawed mental movement representations derived from former improper feedback from a moving body part may not allow for a correct compensation.

Although cerebral compensation mechanisms seem a plausible explanation for the changes in activation patterns after virtual TMS modulation, one has to keep in mind that we did not find any direct effect of TMS under the coil. Even after analyzing all subjects (both patients and controls) in one group, no clear change in activation was seen at or directly aside of the target coordinates. This is not at odds with previous reports from studies with similar TMS parameters in an interleaved setting containing either increased, decreased or no activation at the target site (Bohning et al., 1999; Bohning et al., 2000; Kemna and Gembris, 2003; Sack et al., 2007). Possible explanations can be found in an improper TMS coil positioning or in artifacts of scan slices due to the TMS. Although we used the newest MR-guided positioning system based on the subject's own anatomical scan, visible feedback of the coil position, such as a thumb twitch, remains impossible for the parietal cortex. Nevertheless, we are assured that the TMS-related changes in activation patterns related to the task conditions could not be influenced by an aspecific effect of TMS itself because these (changes in) activations resulted from the comparison with a rest condition that was similarly measured after TMS. Moreover, contrasting the rest conditions after and without TMS did not result in local activations. This indeed allowed for a direct comparison between the task conditions after and without TMS.

To conclude, the results of this study note that the abnormal pattern of activation increases seen in CD could (partly) be explained by an adaptive mechanism of the brain. Impaired anterior parietal function in CD may trigger compensatory activation increases in higher-order motor control regions in prefrontal and posterior parietal cortices in order to still be able to perform the requested movement. Further TMS induced modulation of the left parietal region in CD seems to increasingly challenge these adaptive regions.

Appendix for chapter 5

Table 1 – Subject characteristics

Cervical Dystonia	Gender	Age	Predominant dystonic movement	Additional symptoms	Treatment for dystonia
1	F	48	Left laterocollis	Upper extremity tremor	BTX
2	F	79	Right laterocollis	None	None
3	F	65	Left laterocollis	Generalized dystonia	BTX
4	F	71	Left laterocollis	Upper extremity tremor	BTX
5	F	25	Right laterocollis	None	BTX, trihexyphenidyl, tizanidine, clonazepam
6	M	54	Anterocollis	Spasmodic dysphonia	Trihexyphenydil
7	F	66	Right laterocollis	None	Clonazepam
Mean ± SD		**57 ± 16**			
Healthy Controls					
1 - 10	8F, 2M				
Mean ± SD		**53 ± 11**			

BTX = botulinum toxin

Table 2. Task performance ratings

	Healthy Controls	Cervical Dystonia	Between group difference
Questionnaire (score 48-240)			
movement imagery	109 ± 58	163 ± 63	NS
Speed of performance (s)			
movement execution	23 ± 13	20 ± 9	NS
movement imagery	17 ± 7	34 ± 21	P=0.013
Subjective task rating (1-5)			
movement execution			
practice	4.6 ± 1.0	4.2 ± 1.6	NS
during scan	4.2 ± 1.0 (A)	4.0 ± 1.5 (A)	NS
after scan	4.7 ± 0.9	4.3 ± 1.6	NS
movement imagery			
practice	3.9 ± 1.4	4.2 ± 1.2	NS
during scan	3.8 ± 1.4	3.8 ± 1.2	NS
after scan	4.3 ± 1.1	4.2 ± 1.3	NS

Measurements to assess task performance in healthy controls and cervical dystonia. Subjects had to score their ability to imagine movements (questionnaire). The total score ranged 1-240. During the test of the speed of performance, subjects executed or imagined 10 cycles of right hand flexion/ extension. The total time was measured with a stopwatch. Subjective task rating consisted of a score ranging 1-5 (impossible-normal) that subjects gave for their ability to perform the task. (A) Within-group analysis revealed that movement execution during scanning was more difficult compared to before and after scanning (P=0.037). Note that TMS was preceded in half of the tasks during scanning. It was not possible to let subjects rate their task after TMS compared to no TMS. Therefore, this within-group difference may not be related to TMS.

Table 3. Task performance without TMS compared to rest without TMS

Brain region (Brodmann areas)		Left			Z	Right			Z
		x	y	z	score	x	y	z	score
Movement imagery: Increased activation									
Frontal lobe	Middle f. gyrus (BA46)					34	34	22	3.63
						26	30	18	3.55
	Medial f. gyrus (BA8,6)					8	26	42	4.26
						8	2	60	3.96
	Cingulate gyrus (BA32)	-14	8	42	4.04				
Cerebellum	Anterior					16	-50	-10	3.65
Subcortical	Thalamus	-18	-12	18	3.66				
Movement imagery: Decreased activation: not significant									
Movement execution: Increased activation									
Frontal lobe	Superior f. gyrus (BA10)					28	46	22	4.26
	Middle f. gyrus (BA9)	-34	36	28	3.72				
	Middle f.gyrus (premotor)	-36	-12	66	5.24				
	Medial f. gyrus (preSMA;BA6)					0	14	58	4.35
	Cingulate gyrus (BA32)	-4	-30	38	4.05	0	38	22	4.47
						6	14	42	4.27
	Precentral gyrus (BA4)	-30	-24	54	4.14				
	Inferior f. gyrus (BA47)					34	20	-14	3.91
Subcortical	Thalamus	-14	-10	18	3.93				
	Parahippocampal gyrus	-18	-52	-4	4.27				
	White matter					30	12	32	3.92
Movement execution: Decreased activation: not significant									

Activations resulting from within-group comparisons in cervical dystonia, respectively right hand flexion/ extension execution and imagery compared to rest, without preceding TMS at left superior parietal cortex. Threshold for response height at voxel-level was P<0.001 with subsequent cluster-level correction of P<0.05.

Chapter 6

Structural white matter abnormalities in patients with idiopathic dystonia

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Abstract

We investigated whether structural white matter abnormalities, in the form of disruption of axonal coherence and integrity as measured with DTI, constitute an underlying pathological mechanism of idiopathic dystonia (ID), independent of genotype status. We studied 7 subjects with ID: all had CD as their main symptom (one patient also had spasmodic dysphonia and two patients had concurrent generalized dystonia, both DYT1 negative). We compared DTI images of patients with 10 HC, evaluating differences in mean diffusivity (MD) and fractional anisotropy (FA). ID was associated with increased FA values in the thalamus and adjacent white matter, and in the white matter underlying the middle frontal gyrus. ID was also associated with increase in MD in adjacent white matter to the pallidum and putamen bilaterally, left caudate, and in subcortical hemispheric regions including the postcentral gyrus. Abnormal FA and MD in patients with ID indicate that abnormal axonal coherence and integrity contribute to the pathophysiology of dystonia. These findings suggest that ID is not only a functional disorder, but it is also associated with structural brain changes. Impaired connectivity and disrupted flow of information may contribute to the impairment of movement planning and regulation in dystonia.

Introduction

ID is defined by the presence of involuntary and sometimes painful muscle contractions, resulting in abnormal postures. ID can be classified according to anatomical distribution (focal, segmental and generalized forms), association with specific identifiable genotypes (e.g. DYT1, DYT6), age of onset, or etiology (idiopathic versus secondary forms).

Conventional MRI fails to detect any abnormalities in patients with ID. Therefore, ID is commonly considered a functional brain disorder, without any underlying structural abnormalities. Specifically, it is believed that ID is a disturbance in the initiation and regulation of movements executed by the basal ganglia. In support of this notion, PET studies have demonstrated overactivity of striatofrontal circuit projections in ID (Carbon et al., 2004a). These neuroimaging studies suggest increased glucose utilization in the posterior putamen, globus pallidus, cerebellum, as well as the SMA (Carbon et al., 2004a; Eidelberg, 2003; Galardi et al., 1996). This abnormal pattern is independent of genotype (observed for both DYT1 as well as DYT6 mutations) (Trost et al., 2002) and anatomical distribution (as it is seen in both focal and generalized forms) (Carbon et al., 2004a; Galardi et al., 1996). The abnormal metabolic activity has been demonstrated to persist following the suppression of involuntary dystonic movements by sleep induction (Eidelberg et al., 1998; Hutchinson et al., 2000). These data suggest that dystonia may be associated with an abnormal metabolic resting state that can interfere with motor learning and regulation (Ghilardi et al., 2003).

The notion that ID is a ‘purely functional disorder’ has been recently challenged by studies with post-processed MRI scans, which demonstrated structural abnormalities in patients with ID. For example, manual morphometry studies of the basal nuclei have demonstrated that the putamen is abnormally large in patients with cranial and limb dystonia (Black et al., 1998). A complementary study employing a voxel-wise analysis of gray matter volume of the whole brain found that patients with CD exhibited increased gray matter in the motor cortex, cerebellar flocculus and right globus pallidus interna. In addition, a decrease in

gray matter density was observed in the right caudal SMA as well as in the right dorsal lateral prefrontal and visual cortex (Draganski et al., 2003).

A recent DTI study of symptomatic and asymptomatic DYT1 carriers indicated a reduction in FA within the white matter underlying the sensorimotor cortex (Carbon et al., 2004b). However, it is still unclear whether white matter abnormalities are associated specifically with the DYT1 genotype, or if this finding is universally applicable to all forms of ID.

The present study aims to investigate whether white matter abnormalities are also associated with non-DYT1 forms of ID. We tested the hypothesis that structural white matter abnormalities, in the form of disruption of axonal coherence and integrity as measured with DTI, constitute an underlying pathological mechanism of ID, independent of genotype status.

Methods

Subjects

Patients: We studied 7 subjects with ID (six females, 1 male, age range 25-79 years, mean = 58y ± 18y). All patients had CD as their most prominent symptom. One patient had concurrent spasmodic dysphonia, and two patients had CD in the context of a generalized dystonia (both were negative for the DYT1 mutation). Three patients had left laterocollis, 3 patients right laterocollis and 1 patient anterocollis. Four patients were treated for their dystonia with BTX injections, two patients with trihexyphenidyl, one patient with clonazepam, and one patient with tizanidine. Clinical details are summarized in Table 1.

Controls: Ten controls were enrolled in this study (eight females, age range 26-64 years, mean = 53y ± 11y). There was no difference in age ($t(1)=0.79$, $P=0.437$) and gender (Yates' $Chi(1) = 0.117$ $P=0.732$) between patients and controls. Out of the ten controls studied, seven were age and gender matched with patients (six females, age range 26-64 years, mean = 53y ± 13y). Controls were recruited from the local community and did not have any significant previous medical history, including history of neurological or psychiatric disorders.

All subjects signed a written informed consent to participate in the study, which was approved by the institutional review board of the Medical University of South Carolina.

Imaging

Scanning: All subjects lay in a Philips 3T Intera scanner (Best, Netherlands) using an 8-channel head coil at the Medical University of South Carolina. Routine T1 scans were acquired to investigate for potential presence of structural lesions. DTI images were acquired using single shot EPI with cubic voxels of 2.5 x 2.5 x 2.5mm, employing the default Philips 15 diffusion directions with b value of 1000 s/mm^2 (time of acquisition: 5:40 minutes).

Image processing: We aimed to investigate axonal integrity by performing a voxel-wise analysis of the magnitude (MD) and the directionality (FA) of molecular displacement. DTI images were submitted to pre-processing steps for further voxel-wise evaluation of quantifiable MD and FA. Voxel-wise analysis is performed with images transformed into a common stereotaxic space, where corresponding voxels from each subject represents the same spatial coordinates. We avoided disruption of the original orientation and magnitude of diffusion signal by pre-processing images in an attempt to maintain the original diffusion information, prior to transforming the images to a comparable normalized stereotaxic space.

FSL's (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) Diffusion Toolkit was used to pre-process the diffusion weighted images and construct DTI data. Pre-processing involved the extraction of diffusion gradient directions from DICOM images. Raw images were transformed into Analyze format using MRIcro12. Images first underwent eddy current correction by way of affine transform of each diffusion weighted image to the base b=0 T2 weighted image. This procedure aims to remove the majority of the additional spatial distortion in the diffusion weighted images due to application of diffusion gradients in various directions. Following this correction, it is assumed that all diffusion images acquired of the same slice are in alignment and so a pixel-wise calculation of the diffusion tensor may be performed. Variations in acquisition geometry were corrected and gradients were updated

using the Java applet from the F.M. Kirby Center (www.mri.jhmi.edu/~craig/protocols/dti.html). The pixel-wise calculation of the diffusion tensor is performed using FSL's Diffusion Toolkit, including application of a binary brain mask extracted using FSL's Brain Extraction Tool with fractional threshold of 0.3 to prevent erroneous DTI calculation in the noise background outside the head. This results in calculation of both the MD and FA maps from the DTI data, in the same space as the b=0 image volume of the original DTI acquisition. This b=0 image volume, which is effectively a T2 weighted spin-echo echo planar image, was linearly normalized to a T2 template in stereotaxic space using FLIRT (FMRIB's Linear Image Registration Tool - http://www.fmrib.ox.ac.uk/fsl/flirt/). The same normalization matrix is then applied to FA and MD maps obtained from the diffusion tensor reconstruction step.

FA and MD maps were smoothed to a Gaussian kernel of 8 mm in order to minimize individual variability, improve the normality of data distribution and reduce false positives. Smoothed FA and MD maps were then submitted to comparison targeting differences between patients and controls. This statistical analysis was performed on voxel by voxel basis, i.e., the mean of FA or MD for each voxel was compared between the 7 patients and the 10 controls using a non paired t-test. We employed a software developed in house to perform these voxel-wise comparisons. Our software also made it possible to evaluate each and every voxel contained within a single mask while ignoring the voxels outside of it. Therefore, we aimed to investigate differences in MD and FA within the white matter and gray matter independently. In order to obtain an authentic representation of the location of white and gray matter in FA and maps, we constructed probabilistic maps of the stereotaxic location of white and gray matter, obtained from high resolution T1 weighted MRI with 1 mm isotropic voxels from 80 normal subjects (53 women, age range 17 to 89 years, mean = 30 ± 19y) that were scanned for other studies in the same scanner that the DTI from this manuscript was obtained. These maps (shown in Figure 1) were then registered to the mean of FA and MD maps (from the 17 subjects enrolled in this study: 7 patients and 10 controls). The resulting registered maps were used to construct two masks (one of white and one of gray matter),

which were then used for voxel-wise analyses. Voxel-wise analyses were corrected for multiple comparisons through false discovery rate threshold of 5%.

In order to further refine the investigation of DTI differences between patients and controls, we employed a region of interest (ROI) analysis of MD and FA dissimilarities between patients and controls. This technique differs from the use of masks for voxel-wise analysis. If a mask is used, then all voxels are compared within the mask, and the result is corrected for multiple comparisons based on the number of voxels tested in the mask. During ROI analyses, the mean of MD or FA within the ROI is computed for each individual. This value is then used to compute a mean and SD for that ROI for each group (patients and controls), which are then compared.

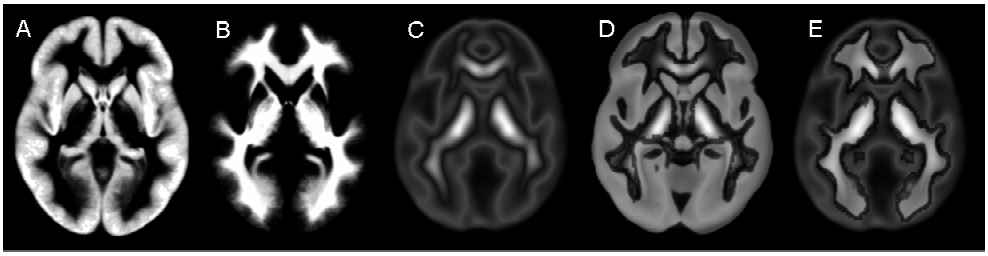

***Figure 1**- Masks encompassing white and gray matter were created for refining voxel-wise FA and MD DTI analyses. Probabilistic maps of gray (A) and white (B) matter were extracted from high resolution T1 scans. These maps were then registered onto the mean FA map (C) from all subjects involved in this manuscript (10 patients and 7 controls). The resulting gray (D) and white matter (E) masks were used as focus areas for DTI analyses.*

ROIs were defined in sites that are expected to be functionally abnormal in patients with dystonia. We used ROIs corresponding to each component of the basal ganglia (thalamus, caudate, putamen and pallidum), and corresponding to supratentorial motor areas: the precentral area and the SMA. ROIs were obtained from the Anatomical Automatic Labeling dataset (Tzourio-Mazoyer et al., 2002) (http://www.cyceron.fr/freeware/). We also aimed to investigate the white matter surrounding the components of the basal ganglia. Therefore, a second set of ROIs was created as follows: each ROI corresponding to a component of the basal

nuclei was dilated employing a 6 mm spatial smoothing and a fractional threshold of 0.05, so that the resulting regions encompassed the basal ganglia as well as the adjacent white matter. The intersections of these areas generated the second set of ROIs, which encompassed only the white matter between different nuclei (one example would be the intersection of the dilated caudate and dilated putamen, which is the anterior limb of the internal capsule). Specifically, we created ROIs that represented the white matter between the caudate and putamen, the caudate and the pallidum, the pallidum and the putamen, the thalamus and the pallidum, and the thalamus and the putamen.

All ROIs were spatially registered to match the shape and space of the mean of MD and FA images. The mean of FA and MD values within each ROI was extracted using the software package MARSbar (Brett et al., 2002). For this ROI analysis, only controls that were age and gender matched with patients were used in order to improve statistical power. Mean differences between patients and controls were calculated using SPSS v12.0 and employing a repeated measures multivariate ANOVA with one between-subjects grouping factor (groups: controls, patients) and one within-subject grouping factor (ROIs). The level of statistical significance was set at $P<0.05$.

Results

Routine T1 MRI scans of dystonia patients and controls were read by a neuroradiologist (N.B.) and found normal in all cases.

Using voxel-wise analyses of voxels contained within the spatial representation of gray and white matter masks, we observed a significant decrease in FA in patients with ID compared with controls. Reduction of FA in patients was located in the gray matter within the posterior portion of the right thalamus and in the white matter in the vicinity of the right thalamus and underlying the right middle frontal gyrus (Figure 2). There were no differences, using voxel-wise analysis of tissue masks, in MD values between patients and controls.

Using ROI analyses of the components of the basal ganglia and of the adjacent white matter, there were significant differences in MD values between patients and

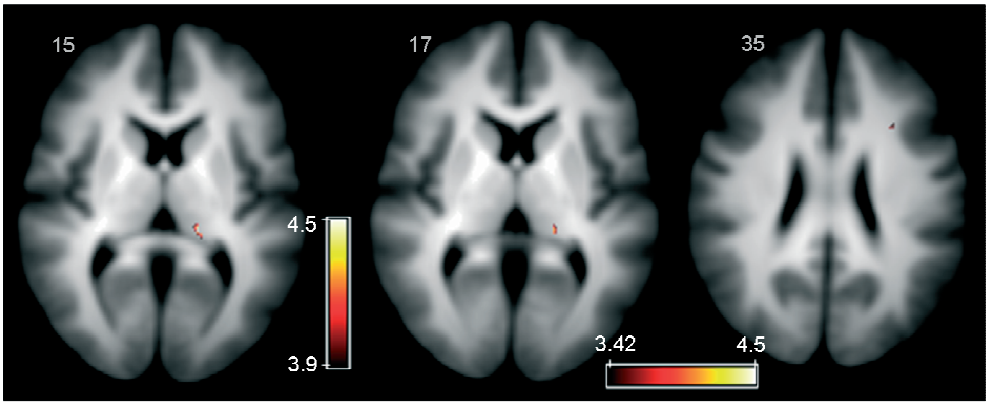

Figure 2 *– Statistical maps of reduced FA in patients with ID compared with controls is overlaid in a T1 template. Stereotaxic z coordinates of the axial slices are displayed in gray. Images are shown in neurological convention (the right side of the image corresponds to the right side of the brain). Scale bars represent Z scores*

controls. A significant increase in MD was observed in patients with ID in ROIs encompassing the basal nuclei and the adjacent white matter, such as the right and left pallidum, right and left putamen, and left caudate (MANOVA λ and p values are outlined in Table 2, and the data distribution is shown graphically in Figure 3). ROI analyses did not show significant differences with regard to mean values of FA for any of the ROIs analyzed, possibly because differences in FA were circumscribed in small regions and were therefore washed out when the mean of a larger ROI was computed.

Discussion

In this study, we used a carefully designed voxel based analysis of molecular displacement within axonal tracts of white matter. This analysis revealed that ID is associated not only with functional brain abnormalities, but also with structural disruption of the normal white matter architecture. Patients with ID demonstrated significant differences with respect to magnitude and directionality of molecular displacement particularly within the basal ganglia, frontal projections and the precentral area.

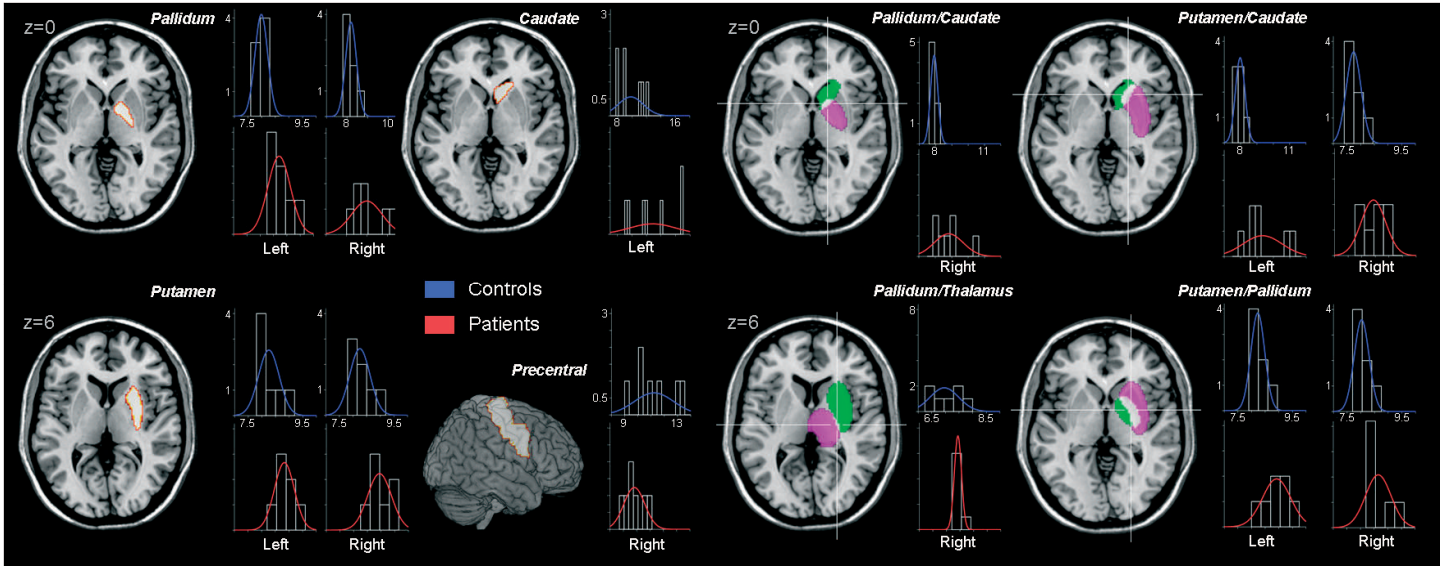

Figure 3 *– Distribution of the mean MD in areas where there was significant difference between patients and controls: within the basal nuclei (pallidum, caudate and putamen), precentral area and basal ganglia white matter (white overlap, highlighted by a cross-hair). Histograms (the x axis denotes the MD value *10^{-4} and the y axis the frequency in number of subjects) depict the distribution of MD for each location (adjacent to its anatomical demonstration). For some structures, differences were bilateral (hence right and left histograms).*

White matter abnormalities have been demonstrated in the sensorimotor cortex in symptomatic and asymptomatic subjects carrying the DYT1 mutation for primary generalized dystonia (Carbon et al., 2004b). However, it is unclear whether white matter abnormalities were exclusively related to the DYT1 genotype, or whether these abnormalities are intrinsically part of the pathophysiology of dystonia. Our data demonstrate that white matter abnormalities are also associated both with focal and generalized forms of ID, independent of the genotype. Compared to the previous DTI study investigating patients with the DYT1 mutation, we studied a more heterogeneous group of patients with different forms of ID. We observed that abnormalities in FA involved not only the sensorimotor area, as previously described for patients with the DYT1 mutation, but also affected other components of the motor circuitry, including the white matter within the basal ganglia. Our findings can be related to improved sensitivity of the methods due to technical advances in DTI acquisition and analysis. First, our data were collected in a scanner with a strong magnetic field (3 Tesla), yielding a higher signal to noise ratio. Second, the diffusion-weighted acquisition utilized 15 diffusion directions that should result in an accurate calculation of FA and MD values (Jones, 2004). Third, we prevented deformation of FA and MD vectors during the transformation of images to equivalent and comparable spatial coordinates for voxel wise analysis; this was done by applying a matrix of linear transformation that was calculated from the transformation of B=0 images.

In addition to confirming the previous DTI results (Carbon et al., 2004b), we were able to demonstrate structural abnormalities in the white matter of the basal ganglia and frontal lobes of patients with ID. These results are in accordance with regional structural abnormalities demonstrated by different structural studies using other modalities of investigation such as gray matter morphometry (Black et al., 1998; Draganski et al., 2003). Moreover, our results concur with functional studies, which demonstrated inappropriate overactivity of striatofrontal projections and impaired activity of motor executive areas in ID (Carbon et al., 2004a; Eidelberg, 2003). Taken together, these data indicate that ID may be caused by impaired sensorimotor integration and disrupted generation and control of movements, due to functional and structural disturbance of cortico-subcortical motor networks.

Further research will be required to understand the full pathological and functional implications of our findings. At the moment, the physiological correlates of abnormal MD and FA signals are speculative. Anisotropy (the opposite of isotropy) refers to being directionally dependent. It signifies that water molecules move preferentially in one direction. Diffusion, on the other hand, quantifies the movement of water particles. Together, these two measurements provide indirect evidence of water molecules transport in the brain (Beaulieu, 2002; Pierpaoli et al., 1996). Because water molecules diffuse in the brain through neural fibers, more specifically through axonal tracts, MD and FA are considered to be indirect measures of axonal integrity. It is possible that areas where FA was reduced in patients with ID represent locations where the molecular movement within axonal fibers was not as organized and homogeneous, therefore corresponding to disrupted connections. Conversely, changes in MD may be related to reduced cellularity (Beaulieu, 2002). Diffusion changes are possibly associated with changes in the space available for extra-cellular water. Thus, an increase in MD is thought to relate to a larger extracellular space.

In a recent innovative study, Colosimo et al. (2005) investigated the differences in FA and MD between CD patients and controls. In their study, the authors observed reduced FA in the white matter of patients with dystonia. However, they observed enlarged FA and reduced MD in the gray matter of the basal ganglia of patients with dystonia. The results from Colosimo et al. are very similar to the results reported in this manuscript, regarding reduced white matter FA in patients with dystonia. However, the results from Colosimo et al. differed from the results of the present manuscript regarding FA and MD values of gray matter. In particular, MD values were reduced in the study by Colosimo et al., but were consistently larger in patients in our study. Interestingly, increased MD is a marker of a reduced number of cells and increased extra-cellular space. Therefore, our findings support the notion of cell loss and impaired connectivity within the white matter of the basal ganglia of patients with dystonia. The differences between our findings and the findings from Colosimo et al. can possibly be accounted by two factors. First, DTI images were different, since we employed images obtained from a scanner with stronger magnetic field (3T), with a larger number of DTI directions (15). Second,

we studied a more heterogeneous group of patients, as Colosimo et al. investigated only patients with torticollis, without concurrent segmental or generalized dystonia.

Overall, the findings from this manuscript and the results reported in the literature indicate that there are significant differences in DTI properties of the brain of patients with ID compared with controls. These findings support current evidence that ID is not only a functional disorder, but it is also associated with structural brain changes. Importantly, our findings demonstrate that DTI abnormalities are not exclusively found in patients with genetically defined generalized dystonia, but can be seen in patients with ID as well. Our findings suggest that patients with ID exhibit an impaired cortico-subcortical connectivity, which may be a cause for the impairment of movement planning and regulation in dystonia.

Appendix for chapter 6

Table 1 - Clinical profile of patients with ID.

Patient	Age	Gender	Race	Most prominent symptom	Additional symptoms	Treatment for dystonia
1	48	Female	Caucasian	Left laterocollis	Upper extremity tremor	BTX
2	79	Female	Caucasian	Right laterocollis	None	None
3	65	Female	Caucasian	Left laterocollis	Generalized dystonia	BTX
4	71	Female	Caucasian	Left laterocollis	Upper extremity tremor	BTX
5	25	Female	Caucasian	Right laterocollis	None	BTX , trihexyphenidyl, tizanidine, clonazepam
6	54	Male	African-American	Anterocollis	Spasmodic dysphonia	Trihexyphenydil
7	66	Female	Caucasian	Right laterocollis	Generalized dystonia	Clonazepam

BTX = botulinum toxin

Table 2 – Regions of interest

Region of interest	Λ	Sig.
right pallidum	15.25	0.002
right caudate/putamen	11.89	0.005
left putamen	10.18	0.008
left pallidum/putamen	9.91	0.008
right pallidum/putamen	9.17	0.010
left caudate/putamen	8.67	0.012
right putamen	8.52	0.013
left pallidum	7.15	0.020
right caudate/pallidum	6.82	0.023
right pallidum/thalamus	5.03	0.045
left caudate	4.90	0.047
left precentral	4.75	0.049

Regions of interest analyses for greater MD in patients compared to controls. The white matter between two nuclei is represented by nuclei1/nuclei2.

Chapter 7

Cerebral activation patterns related to initiation and inhibition of hand movement

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Abstract

Sequential ordering of purposeful movements includes distinct transitions between muscle contraction and relaxation. To explore cerebral activation patterns underlying such movement initiation and inhibition, we applied fMRI to test the effects of (i) ballistic movement (dominated by initiation), (ii) movement with stepwise interruption (dominated by inhibition) and (iii) smooth movements. Right-hand movements were performed by 21 healthy subjects. In the basal ganglia, ballistic movements evoked putamen activation, indicating its specific contribution to initiation. Stepwise interrupted movement induced increased activation of the caudate nucleus, globus pallidus and STN while, at the cortical level, SMA activation increased. This indicates a specific basal ganglia-thalamocortical circuit involved in motor inhibition.

Acknowledgement

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Introduction

With regard to the cerebral control of upper limb movement, one might distinguish at least two levels of organization. Task-related movement such as grasping, requires a high degree of visuomotor integration (de Jong et al., 2001). On the other hand, the orchestration of fine-tuned muscle contractions demands a balance between agonist and antagonist muscle contractions, force estimation and sharp transitions between muscle contraction and relaxation (Aron and Poldrack, 2006). This orchestration enables the precise onset and ending of movement. Disturbed task-related movement is particularly seen in the apraxias, generally associated with parietal and premotor cortex lesions (Rushworth et al., 2003). In extrapyramidal movement disorders such as Parkinson's Disease and dystonia, initiation and inhibition of movement are compromised (Berardelli et al., 1998; Hallett, 1990; Naumann et al., 2000). Dysfunction of basal ganglia, prefrontal cortex and the SMA have been implicated in these diseases (Abbruzzese et al., 2001). The distinction between task-related movement and basic commands for muscle contraction is also reflected in paradigms that are used in functional imaging studies that have addressed the functional anatomy of cerebral motor control (Blood et al., 2004; Ceballos-Baumann et al., 1995; Delmaire et al., 2005; Grezes and Decety, 2001).

In the present fMRI study, we sought to identify cerebral regions particularly involved in the initiation and inhibition of simple hand movements in healthy subjects. To that end, we designed a paradigm that included ballistic, stepwise interrupted and smooth flexion- extension movements that were made either in the wrist or with the fingers. We hypothesized that particularly the SMA and basal ganglia structures are involved in the non-smooth movement tasks. In this respect, we aimed to create a paradigm that can be used in future research on extrapyramidal movement disorders.

Methods

Subjects

Twenty-one right-handed subjects [age range 23-47: mean 32 ± 7 (SD), male=11] successfully participated in this fMRI study, approved by the Institutional Review Board, Medical University South Carolina. Informed consent was based on the Helsinki Declaration. There was no conflict of interest in this study. Subjects were questioned about profession, hobbies and handedness (Annett, 1970). They had no history of neurological or psychiatric disease.

Behavioral task

Six right-hand movement patterns were performed, subdivided into wrist and fingers tasks. To investigate differences between initiation and inhibition of movement, our paradigm included smooth movement, ballistic movement and stepwise interruption of movement. Smooth movement had a gradual movement trajectory and served as control condition. Ballistic movement particularly involved abrupt initiation and was physiologically limited by the maximum excursions of the target joint. Stepwise interrupted movement consisted of a repeated flexion/ extension cycle of alternating initiation and inhibition. This condition contained 4 evident stopping elements. Inhibition was extracted from stepwise interrupted movement minus the initiation task. Each movement cycle was performed within a period of 2 seconds.

1) For wrist movements, the right hand was positioned in a vertical plane, thumb on top, in a flexed position. The excursion started with extension to the right followed by flexion back to the initial position. Fingers and thumb were parallel. The randomly performed tasks were: (i) a smooth movement cycle from the most distant flexed position to the most distant extended position and back, (ii) brisk movement from the flexed to the extended position, followed by a brisk return movement (wrist ballistic) and (iii) brisk movements, now with additional stops halfway the excursion, at the most extended position, halfway returning to initial flexion and at initial position (wrist stepwise).

2) Finger movements according this threefold concept were made in the metacarpo-phalangeal and proximal interphalangeal joints: (i) smooth movement with flexion of the fingers towards the hand palm and back to extension, (ii) fingers ballistic and (iii) fingers stepwise. Additional flexion in proximal interphalangeal and distal phalangeal joints was allowed, making a fist, however, was not the intention. A rest condition followed each movement block.

Practise was during the screening session and just before scanning. Visual displays instructed the subject when and how to perform by an abbreviation of the task and a moving bar. Visual presentation was accomplished with an in-scanner display (Integrated Functional Imaging System, IFIS, Invivo, Orlando, FL, USA). The start of the paradigm was triggered by the first pulse from the scanner.

fMRI method

Subjects were scanned at the Charleston Medical University of South Carolina, using a 3 Tesla MR scanner with a multi-channel head coil (standard SENSE coil, Philips Medical Systems, NL). T2* weighed, three-dimensional functional images were acquired using principles of multi slice echo planar imaging sequence (EPI). 36 slices (3 mm slice thickness) with a 64*64 matrix were acquired using the following parameters: TR = as a volume in a TE (echo time)=1.8 seconds, TE=30 ms, number of volumes = 515 voxel size 3.5x3.5x3.0 mm, total scan time = 16 minutes. A set of T1-weighted images (1.0x1.0x1.0 mm) enabled the definition of anatomical regions.

Subjects were laying supine, head fixated in the head coil with foam pads. Noise was partially masked using earplugs. The tasks were performed in a fixed randomized block-design, containing 20s. blocks with each 5 movements. An online connected flexible armrest supported the arm and measured the angles of joint excursions, enabling the verification of task compliance.

Data analysis and statistical methods

The analysis was performed using FMRIB Software Library (FSL, Oxford, UK, www.fmrib.ox.ac.uk/fsl), for image realignment, normalization and statistical

analysis of fMRI data. Lower level analysis was carried out using FEAT (FMRI Expert Analysis Tool) Version 5.63, part of FSL. Pre-statistics processing included motion correction using MCFLIRT (Jenkinson et al., 2002), non-brain removal using BET (Smith et al., 2002), spatial smoothing using a Gaussian kernel of Full Width Half minimum (FWHM) 8mm, mean-based intensity normalisation of all volumes by the same factor, highpass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma=50.0s). Time-series statistical analysis was carried out using FILM with local autocorrelation correction (Woolrich et al., 2001). Z (Gaussianised T/F) statistic images were thresholded using clusters determined by Z>2.3 and a (corrected) cluster significance threshold of P=0.05 (Worsley et al., 1992). Registration to high resolution and/ or standard images was carried out using FLIRT (Jenkinson and Smith, 2001).

Higher level analysis was carried out using FLAME (FMRIB's Local Analysis of Mixed Effects) stage 1 only (without the final MCMC-based stage) (Beckmann et al., 2003; Woolrich et al., 2004), Z (Gaussianised T/F) statistic images were also thresholded using clusters determined by Z>2.3 and a (corrected) cluster significance threshold of P=0.05. Localisation of (cluster) maxima was obtained by translating MNI coordinates to Talairach coordinates in Matlab (Mathworks, Matlab and Simulink).

Region of Interest Analysis (ROI)

Because of the practical use of our paradigm in future studies on extrapyramidal disorders, we were particularly interested in effects in relatively small subregions of basal ganglia and thalamus. Therefore, separate masks for both basal ganglia and thalamus were created by drawing the basal ganglia and thalamus manually in a 3D binary volume of interest, following the hypointense regions and rendered on the MNI template, using MRIcro Software (C. Rorden, University of South Carolina, Columbia, USA). These pre-threshold masks constrained our search for activation in basal ganglia and thalamus and reduced the number of voxels tested which made multiple-comparison-correction less stringent. Only voxels containing zero in the mask images would be zeroed in this process. After masking, a normal threshold procedure was maintained with P= 0.05 (uncorrected) voxel level (FSL).

Results

Smooth, ballistic and stepwise inhibition; whole brain analyses

Contrasting each of the 6 movement conditions to rest resulted in activation of hand-associated circuitry, including the contralateral sensorimotor cortex, extending into the (dorsal) premotor cortex. In the contrast 'smooth versus ballistic' as well as 'stepwise versus ballistic', the sensorimotor cortex showed significantly increased activation. Additional activation was more obvious in the contrast that extracted inhibition, 'stepwise versus ballistic'. Comparisons between the three types of movements did show subtle differences when made either by the fingers or by the wrist. As we were particularly interested in the general aspects of initiation and inhibition, finger and wrist movements were not further distinguished and treated as a single group in all following analyses. To isolate initiation in movement, ballistic movement was contrasted to smooth movement. This resulted in activation (i) of left anterior insula-and frontal operculum, (ii) of the left dorsolateral prefrontal cortex (the anterior junction of the premotor cortex), (iii) along the intraparietal sulcus, and (iv) along the superior temporal sulcus bilaterally. 'Ballistic versus stepwise' showed similar activation in the insula and frontal operculum, bilaterally, and anteriorly along the right intraparietal sulcus. Unique for the ballistic movement was the activation of the insula and frontal operculum. Unique cortical areas related to inhibition, identified by comparing stepwise interrupted movement to the other tasks, were SMA ('stepwise versus ballistic') and pre-SMA ('stepwise versus smooth') (Figures 1, 2).

Basal ganglia

Using the basal ganglia mask, all conditions versus rest activated bilateral putamen and globus pallidus. Unique for ballistic movement, compared to smooth as well as stepwise interruption, was the strong contralateral (left) putamen activation. In the opposite contrast, 'stepwise versus ballistic', the caudate nucleus and globus pallidus were activated. During 'stepwise versus smooth' left putamen was also activated, but less robust than the result of comparing ballistic with smooth movement (Figures 1, 2).

Thalamus

All conditions versus rest activated the thalamus bilaterally, masked with a thalamus ROI. Ballistic movement had only minor effects when contrasted to the other movement conditions: posterior thalamus activation was seen in comparison with smooth movement, while the comparison with stepwise interruption did not result in a significant effect. The contrast 'stepwise versus smooth' resulted in a robust activation of the location which best fits the subthalamic nucleus, bilaterally, together with left medial thalamus activation. The subthalamic nucleus activation was also seen after contrasting 'stepwise versus ballistic', although restricted to the left side. Thus, activation of the subthalamic nucleus was only seen in the stepwise interrupted movement (Figures 1, 2).

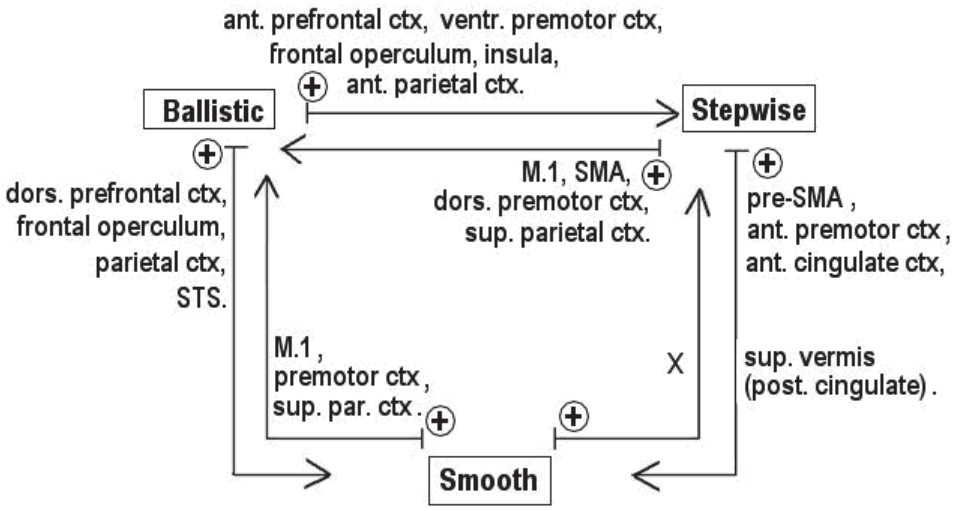

Figure 1 - Scheme of the Contrasted Conditions (combination of wrist and fingers)

Relation between the 3 movement conditions and the areas that were significantly activated during the different contrasts are shown. The arrows point out in which direction the contrasts are to be read (e.g. 'ballistic → stepwise' = 'ballistic versus stepwise'). The cross signs indicate areas that were more activated during that movement, regarding the adjacent contrast. (ctx = cortex)

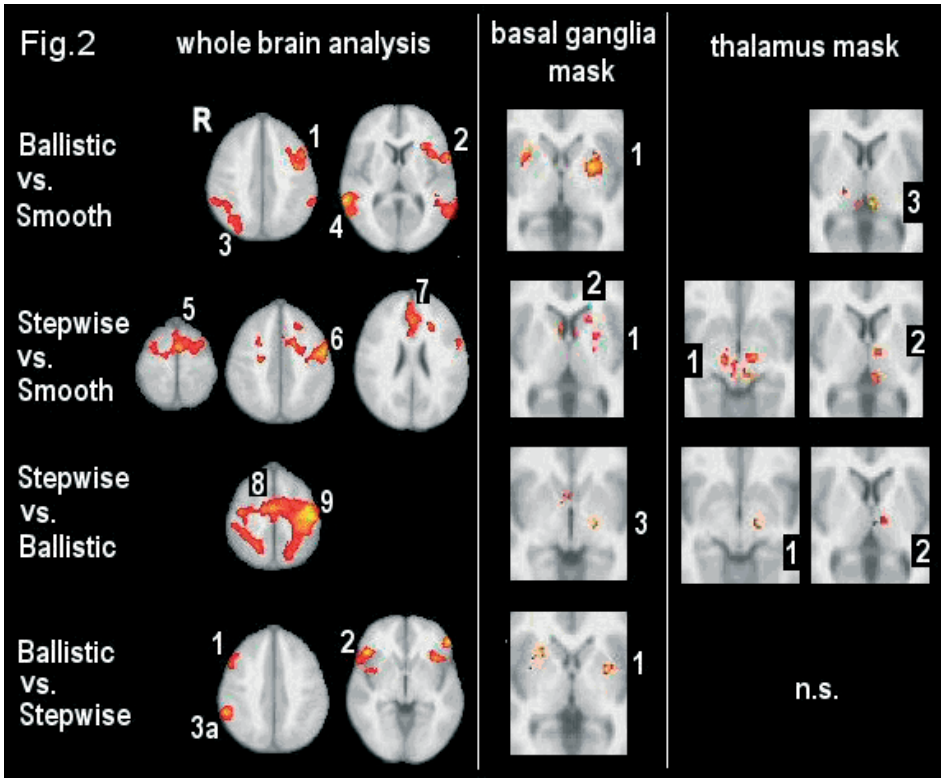

Figure 2 - *Summary of main results (combination of wrist and fingers)*

Clusters of activity are rendered on the MNI template displayed on transversal sections (left side is right, marked 'R'). For the whole brain analysis, data was thresholded at P< 0.05, cluster level corrected. The x,y,z coordinates correspond with the relation to the middle of the anterior commisure. Threshold for the masked images was P< 0.05, uncorrected voxel-level. Here, only the z coordinate is given to indicate the position of the transversal plane relative to the AC-PC plane. The numbers correspond to the following activated cerebral regions:

Whole brain analysis:

1= dorsolateral prefrontal cortex (ctx) / junction anterior premotor ctx: -46, 2, 42 / 55, 21, 23 mm; 2= frontal operculum, ant. insula: 32, 21, -3 mm: -20, 45, 5 mm; 3= intraparietal sulcus: 36, -60, 47 mm; 3a= ant. intraparietal sulcus: 53, -52, 47 mm; 4= superior temporal sulcus: 65, -58, 14 mm; 5= (pre-) SMA: 16, -6, 65 / -6, 9, 70 mm; 6= ant. premotor ctx: -44, -2, 46 mm; 7= ant. cingulate ctx: -4, 53, 8 mm; 8= SMA; -26, -22, 62 mm; 9= primary motor area: -42, -11, 54.

Basal ganglia mask: *1= putamen (z= 0 / 8 mm); 2= caudate nucleus (4 mm); 3= globus pallidus (-4 mm).*

Thalamus mask: *1= subthalamic nucleus (z = -8 mm); 2= ant. thalamus (4 mm); 3= post. thalamus (4 mm).*

Discussion

With the employed paradigm we aimed to selectively challenge the cerebral organization of movement initiation and inhibition with a particular focus on the role of the basal ganglia and SMA in the non-smooth movement tasks. The basal ganglia play a crucial role in the initiation and execution of voluntary movements as well as inhibition of competing movements (Alexander and Crutcher, 1990). The SMA is particularly involved in movement planning and timing, which implies a more complicated orchestration of initiation and inhibition (Rizzolatti and Luppino, 2001).

Consistent with our hypothesis, we found less cortical activation during ballistic movements, while putamen activation was strong. The primary motor area was particularly active in the non-ballistic conditions. This dominance of striatal involvement together with a limited motor cortex contribution supports the characteristics of an initiation task. Ballistic movement is particularly characterized by the initiation of movement, without prominent regulation during the successive movement excursion, while the ending of movement is caused by restrictions of the involved joint. This explains why the sensorimotor cortex activation is particularly strong in the opposite conditions. Indeed, both smooth and stepwise interrupted movement demand an increased regulation of efficient motor commands during excursion over the joint. The use of more muscles and prolonged contractions is thus reflected by increased activation of M1 and adjacent premotor and sensory cortical regions. Complementary to these movement-related cortical activations, the strong putamen activation contralateral to the ballistic movement points at relative simple features of this movement pattern: a brisk contraction of a few muscle groups with maximal relaxation of their antagonists.

During stepwise interrupted movements, the premotor cortex and SMA were significantly more active than in the other conditions, from which we infer their particular involvement in inhibition. In the basal ganglia, stepwise movement exhibited the unique feature of subthalamic nucleus and globus pallidus activation. The latter indicates a contribution to muscle relaxation (de Jong and Paans, 2007),

while functional cooperation of globus pallidus and subthalamic nucleus fits existing concepts (Plenz and Kital, 1999).

Only few prior imaging studies have addressed initiation and inhibition of movements. Ipsilateral inferior frontal cortex, subthalamic nucleus and SMA have been implicated in response inhibition by using a stop task. The right inferior frontal cortex was thought to excite the subthalamic nucleus by which it suppresses the basal ganglia thalamo-cortical output. SMA involvement in inhibition has further been concluded from rhythm paradigms (Lewis et al., 2004).

The background of this study was focused on extrapyramidal disorders as Parkinson's Disease and dystonia, in which initiation and inhibition are compromised. Based on our results, we recommend using an inhibition/ initiation paradigm as described in this paper to investigate these disorders.

Conclusion

Complex alternation of initiation and inhibition involves (pre-)SMA, premotor and sensory cortical regions, while 'simple initiation' requires more involvement of the putamen.

Appendix for chapter 7

Table 1

Brain region (Brodmann areas)		Left			Z	Right			Z
		x	y	z	score	x	y	z	score
(wrist + fingers ballistic) vs (wrist + fingers smooth)									
Frontal lobe	Middle (BA6)	-46	2	42	3.65				
	Inferior (BA47)	-32	21	-3	3.63				
	Precentral gyrus (BA44)	-57	10	7	3.49				
Temporal lobe	Superior (BA22)	-59	-48	17	4.29	63	-40	8	4.08
						65	-58	14	3.42
	Middle (BA37)	-63	-66	35	2.93				
		-51	-43	35	2.69				
Parietal lobe	Superior (BA7)					36	-60	47	3.21
	Supramarginal gyrus (BA40)	-59	-41	37	3.27				
(wrist + fingers stepwise) vs (wrist + fingers smooth)									
Frontal lobe	Middle (BA10)	-6	51	5	3.78				
		-4	53	8	3.71				
	Medial/ SMA (BA6)	-6	9	70	3.56	16	-6	65	3.68
		0	5	62	4.06				
	Primary motor area	-44	-2	46	4.37				
(wrist + fingers stepwise) vs (wrist + fingers ballistic)									
Frontal lobe	Precentral gyrus (BA6,4)	-40	-5	52	4.83				
		-42	-11	54	5.29				
		-42	-15	50	5.21				
		-26	-15	62	5.20				
		-28	-12	60	5.06				
(wrist + fingers ballistic) vs (wrist + fingers stepwise)									
Frontal lobe	Middle (BA47,10)	-51	39	-5	3.79				
		-44	43	13	3.12				
		-34	49	12	3.10				
	Medial/ anterior cingulate	-20	45	5	2.98				
	Inferior (BA47)					57	17	-3	3.40
						53	22	12	3.40
						55	21	23	3.32
Temporal lobe	Middle (BA22)					65	-41	4	2.90
Parietal lobe	Superior (BA7)					38	-59	58	2.89
	Inferior (BA40)					63	-42	24	3.05
						53	-52	47	2.96
	Supramarginal gyrus (BA40)					57	-39	33	3.72
						61	-49	25	2.85
Subcortical	Extra-Nuclear, grey matter	-36	19	-3	3.47				

Columns show Talairach coordinates (x, y, z) in mm indicating the location of the activated clusters in relation to the anterior and posterior commisure (AC-PC) plane, Z-score of local maximal peak activation, laterality and the corresponding anatomical loci on the Talairach canonical brain.

Chapter 8

Summary and Discussion

Discussion

The theme of this thesis concerns the cerebral organization of hand movement in CD patients, with special attention to sensory processing necessary for movement preparation. We implemented various methods to tackle our research questions. Besides the more commonly applied fMRI and EMG methods, interleaved TMS/fMRI and DTI were employed as useful new techniques with major conceptual advantages and were expected to add new information to the existing thoughts and theories about dystonia.

Abnormal cerebral activation patterns

Abnormal cerebral organization of clinically normal hand movement in CD was demonstrated by abnormal cerebral activation patterns (***chapter 2***). During movement execution, reduced activation was found in right putamen, insula and cingulate cortex with an activation increase in the right prefrontal cortex. Movement imagery (which may be equated with movement preparation) showed reductions of activation in superior parietal cortex and S2, left premotor cortex, cingulate cortex, putamen and insula. We conclude from these results that circuitry involved in sensorimotor integration is affected in CD. This abnormality is especially present during movement preparation, since only movement imagery showed reduced activation in the mentioned regions. Since movement imagery does not involve sensory feedback during movement performance, abnormal activation during this task provides support for the notion that CD concerns a movement preparation disorder and not a disorder of movement performance evaluation. Impairments in sensorimotor integration in the parietal cortex during movement preparation and planning may lead to impaired activation of interconnected cortical areas as the premotor cortex. This was reflected in our study by reduced activation in left premotor cortex and cingulate gyrus. M1 involvement was not seen during imagery compared to execution. This finding is consistent with some prevous studies (Binkofski et al., 2000; Boecker et al., 2002; Gerardin et al., 2000; Naito et al., 2002) although a number of other studies did find contralateral M1 activation during an imagery task (Lotze et al., 1999; Rodriguez et al., 2004; Sharma et al., 2008;

Solodkin et al., 2004). This discrepancy may be explained by methodological differences as suboptimal subject numbers for whole brain voxel-based analysis or variances in imagery tasks. Tasks that are imagined to be carried out in limbs that have large motor cortex representations, such as finger movements, are more likely to implicate M1 activation (Rodriguez et al., 2004; Sharma et al., 2008) than more proximal arm tasks (Binkofski et al., 2000) or imagined wrist movements (Naito et al., 2002). The type of task may also influence M1 activation. In ***chapter 7***, ballistic movement execution has shown more M1 activation than smooth movement execution, although we can only speculate whether imagery of ballistic or smooth movements will show such differences as well.

When we compare our results with the existing literature on dystonia, it is interesting to notice that in our study only movement imagery showed impairment in regions related to sensorimotor integration, while most other fMRI studies reported these activation changes ***during*** task execution (Ibanez et al., 1999; Odergren et al., 1998; Preibisch et al., 2001). This difference can be explained by the fact that almost all studies in dystonia obtain cerebral activation during an execution task in the affected body part whereas in our study subjects performed the task in a clinically normal hand. We postulate that during execution of a clinically normal movement sensory feedback is sent back from the moving limb to the involved sensorimotor region thus providing an update that normalizes the impaired capacity for sensorimotor integration. Only two other studies used an imagery task in dystonia. In a PET study, patients with idiopathic torsion dystonia activated similar cerebral areas compared to controls during movement imagery of freely selected joy stick movements (Ceballos-Baumann et al., 1994). A recent fMRI study of our group used an identical movement imagery task in the affected hand of dystonia patients with complex regional pain syndrome. Here, reduced activation was present ipsilaterally in the premotor and adjacent prefrontal cortex, and in a cluster comprising frontal operculum, the anterior part of the insular cortex and the superior temporal gyrus. Contralaterally, reduced activation was seen in the inferior parietal and adjacent primary sensory cortex (Gieteling et al., 2008). Although these results partly overlap with ours, the imagery task was again

focused on the affected body part and could therefore not be fully extrapolated to our results.

Activation changes were not only found cortically, but also subcortically in the basal ganglia. Basal ganglia are intimately connected to cortical function and are particularly important for movement initiation and inhibition. The latter are both affected in dystonia. The speed of movement initiation is significantly slowed in dystonia and is consistent with clinical reports of bradykinesia and slowness of movement (Jahanshahi, 2001; van der Kamp, 1989). Reduced inhibitory control of the basal ganglia towards M1 induces a loss of selectivity in the motor output, resulting in muscle overflow and co-contractions during movement in a dystonic body part (Berardelli et al., 1998). Neuroimaging studies show discrepancies in changes of basal ganglia activation in focal dystonia. Basal ganglia activation increases were seen in focal hand dystonia (Blood et al., 2004) and writer's cramp (Hu et al., 2006), while other studies in focal hand dystonia (Delmaire et al., 2005) and hand dystonia combined with chronic regional pain syndrome (Gieteling et al., 2008) reported activation decreases. Our results in CD also show reduced activation in the basal ganglia. Only the study of Blood and colleagues, using an alternating finger-tapping task, and our study looked at basal ganglia activation in relation to movement of a non-dystonic body part, and found opposite results indeed. The increased complexity of the task used by Blood et al. may have played a role. Moreover, we recognized the fact that the smooth flexion/ extension movement task in our study was not designed to optimally focus on differences in movement initiation and inhibition in relation to basal ganglia activation changes. In this respect, we aimed to improve our paradigm and therefore set up a new study to investigated different hand movement tasks (**chapter 7**). The ballistic movement represented initiation, while the stepwise and smooth versions contained a complex alternation of initiation and inhibition. The results showed that movement preparation seems to be more related to initiation, with particular frontal and intraparietal sulcus involvement, while regulation during movement is related to inhibition and requires sensory information and extensive premotor and basal ganglia activation. In this respect, future studies focusing on movement preparation might want to consider ballistic movement tasks while movement performance may

be better reflected in stepwise movement tasks. For dystonia, movement inhibition is more impaired than initiation (Berardelli et al., 1998; Blood et al., 2004), so the stepwise task appears to be most suitable. The smooth movement task is compatible with the stepwise task but activations are less strong.

Compensation mechanisms

Reduced activations in cortical and subcortical regions were seen during execution and imagery of a clinically normal hand movement. The association of reduced activation in movement preparation related cerebral regions and clinically normal movement performance implies effective compensation. A perfect candidate to compensate impaired preparatory regions could be the prefrontal cortex, an area that is well connected with the anterior parietal and premotor areas (Cipolloni, 1999). A trend towards increased prefrontal activation was indeed already seen in CD (***chapter 2***). To test possible cerebral adaptive mechanisms for impaired sensorimotor integration, we set up an interleaved TMS/fMRI study. One part of the study looked at the cerebral network activation changes after TMS-induced disruption of the left superior parietal cortex in HC. We hypothesized that TMS-induced disruption of this region in HC would result in similar activation patterns as seen in CD (***chapter 4***).

The choice to target the left superior parietal cortex was based on the abnormal activations of this region in CD that was thought to reflect impaired sensorimotor integration (***chapter 2***). Its involvement in CD is consistent with the observation that a parietal lesion in the same region induced a dystonic head tremor (Kim and Lee, 2007). The target coordinates were part of a cluster that was partially located on the outer regions of the cortex. Superficially located activation is relevant since TMS can only penetrate a few centimeters into the cortex. One may oppose that it might have been more relevant to focus TMS to S2 itself. By targeting the S2 area, a direct disruptive effect on S2 could have been measured and any compensation mechanisms may be more closely related to impaired sensorimotor integration than is the case now. Moreover, it would be interesting to see what effect TMS-induced disruption of S2 would have on activation during movement execution. Stimulating more anteriorly than the current target area would be in line with a PET study in

which focal dystonia symptoms temporarily improved after applying a sensory trick, reflected by a trick-related increased parietal activation that lay further anterior than our target area (Naumann et al., 2000). Having applied TMS to that region, we would at least have known from literature that the area could be manipulated (i.e. sensory tricks changed cortical activation) and that TMS targeted to that area may potentially improve symptoms. On the other hand, there are advantages of the current TMS target. By choosing the current target area, we were sure that this region was affected in CD since the activation was reduced in the patient group compared to HC. Also, the current target area had only indirectly an impact on S2. This indirect S2 modulation permitted us to investigate the normal adaptations of the network directly connected to S2. Notwithstanding the group results, variable activation patterns may occur in each individual subject. We have to consider the fact that each individual has its own optimal target area which is not exactly the same area as was used now or that the recruitment of cerebral adaptation mechanisms in response to TMS modulation may be different between subjects. All in all, the choice of the current target region has its pros and cons.

The TMS-induced disruption of the left superior parietal cortex during movement execution and imagery in HC resulted in increased bilateral prefrontal and temporal/ posterior parietal cortex activation with decreases in anterior parietal cortex during movement imagery. This TMS-induced cerebral activation pattern in HC was similar to cerebral activation patterns in CD (without TMS) (***chapter 2***). We propose that the compensation mechanisms of the brain follow the hierarchical organization of cortical areas involved in intentional movement (Figure 1). “Simple” (automated) movement execution tasks are coordinated by the primary areas such as M1 together with the basal ganglia. More complex movements require more preparatory information from secondary areas such as premotor and anterior parietal cortices (S2) or even tertiary areas such as posterior parietal and prefrontal cortices in order to perform the desired action (Fang et al., 2005; Johnson et al., 1996; Rizzolatti and Luppino, 2001). Previous TMS studies provide support for this concept by demonstrating that virtual lesioning of M1 induces dorsal premotor activation, while a disturbing TMS effect of the dorsal premotor cortex increases cingulate cortex and SMA activation (Lee et al., 2003; O'Shea et al., 2007).

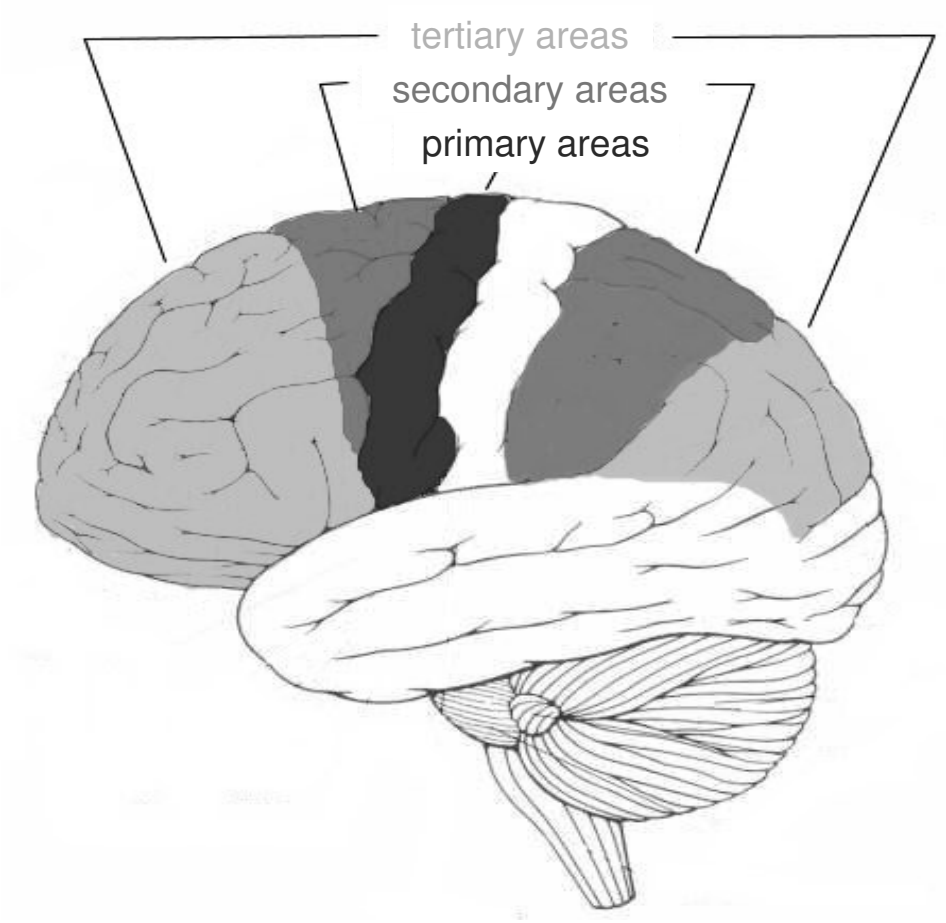

Figure 1 *– Schematic figure of the hierarchical organization of cortical areas involved in intentional movement. "Simple" (automated) movement execution tasks are coordinated by the primary areas such as M1 and basal ganglia. More complex movements require more preparatory information from secondary areas such as premotor and anterior parietal (S2) or even tertiary areas such as posterior parietal cortex and prefrontal cortex in order to perform the desired action.*

Moreover, TMS-induced manipulation of the dorsolateral prefrontal cortex leads to robust activation increases in prefrontal and parietal cortices (Rounis et al., 2006). This principle has also been demonstrated in patients recovering from a hemiparetic stroke. Compared to controls, patients showed increased activation during a simple hand movement task in the M1/ S1 contralateral to the lesion. Linear to the increase of task complexity, additional activations were seen in

secondary areas and even tertiary areas in the patient group (Schaechter and Perdue, 2008). Translating this to our study, TMS-induced disturbance of the left superior parietal cortex challenges tertiary areas within the motor control network (prefrontal and posterior parietal cortex) to gain access to alternating pathways going downstream to the motor cortex, still enabling the preparation of a given movement.

In the second part of the interleaved TMS/fMRI study, we applied this strategy in CD (***chapter 5***). We wondered what would happen to already existing cerebral compensation mechanisms evoked by the disorder-related impairments of a secondary area when the latter would be stressed further with TMS. During movement imagery in CD, TMS was related to a subthreshold activation increase in the left medial frontal cortex with reduction in dorsal premotor regions. A slightly different pattern was seen during movement execution with a left inferior parietal and right prefrontal cortex activation increase, while no increase in posterior parietal activation was found. Although the results are not as manifest as in the HC study, they are coherent with our proposed hierarchically based adaptive strategy of the brain following impaired cortical function. It seems that in CD further cerebral adaptations to impaired cortical function is still possible, although to a lesser extent than in HC. In CD, such adaptations were still successful since a clinically normal movement was achieved.

An interesting follow-up topic for the present study would be to ***stimulate*** the left superior parietal cortex (or S2) with a frequency above 1 Hz instead of the inhibitory 1 Hz frequency that was currently used for inducing functional disruption. Would such enhanced stimulation bring about an increase in activation at the target area? And if so, will it lead to a “normalization” of the cerebral activation patterns, and ultimately relieve dystonia symptoms? TMS has already proven to be a therapeutic tool for patients with depression and was recently approved by the Food and Drug Administration in the United States as an official treatment option in depression (O' Reardon et al., 2007). Promising results of a TMS study in writer’s cramp showed that this technique might also be a treatment option for dystonia in the future (Siebner et al., 1999b).

TMS

Besides the fact that the obtained results gained support for a compensation mechanism theory in CD, the two interleaved studies also illustrate that interleaved TMS/fMRI on the parietal cortex is safe and feasible. The exact mechanism of TMS modulation of cortical function is still pending. However, one of the first interleaved TMS/MRI studies showed that the field of a TMS coil could be mapped with MRI. This mapping was done with a figure-of-eight coil which consists of two circular coils mounted adjacent to each other in the same plane with currents circulating in opposite direction. With this configuration, the fields of the two loops come together at the intersection in the middle of the coil and create a cone-shaped volume of a concentrated magnetic field. This magnetic field can penetrate the brain to a maximum depth of 10 cm. The peak of the strength is between 2-3 cm and drops off rapidly with distance away from the coil (Bohning et al., 1997; Bohning et al., 2001). This peak magnetic field strength is hypothesized to induce an electric current that in turn polarizes the membrane of neurons. This is followed by an action potential which can travel long distances to remote areas. Considering the presence of this local TMS-induced magnetic field, it is questionable why our data lacked a measurable local TMS effect. Although some interleaved TMS/fMRI studies also have not found a local TMS effect after stimulation of the lateral premotor cortex, M1 and parietal cortex (Baudewig et al., 2001; Bohning et al., 1999; Kemna and Gembris, 2003), other studies have observed activation changes when targeting M1/S1 and prefrontal cortex (Bestmann et al., 2003; Bohning et al., 1998; Nahas et al., 2001). It has been noticed that TMS induces an fMRI signal reduction under the coil that may reduce the statistical significance of activation in the target area (Bohning et al., 1999). Possibly, such signal reduction represents a still unclear neuronal inhibitory effect of TMS (Bohning et al., 1999) or modulation of the input from afferent projections to the cortex (Baudewig et al., 2001). In this respect, we may have to consider the possibility that the net effect of TMS may not be primarily mediated by a local change in activation (Baudewig et al., 2001), but with more distributed changes in local neuronal activity that are not associated with the local changes in cerebral perfusion.

Pre-dystonic state

In focal dystonia, dystonic involvement is clinically only seen, by definition, in one part of the body, although other parts of the body may exhibit subclinical dystonic movement abnormalities (Carboncini et al., 2004; Quartarone et al., 2008). These subclinical abnormalities may be relevant because it suggests that dystonia may be a generalized movement disorder with hereditary or environmental traits. To further investigate this phenomenon, we conducted two experiments in which movements were executed in a clinically normal, non-dystonic body part. Wrist flexion/ extension movements in CD patients provided an ideal combination, since these movements are easy to quantify with EMG and can be executed in the MR-setting without movement artifacts of the MR images. In the EMG study (***chapter 3***), we found less effective muscle activation in CD patients while they performed clinically normal hand movements. Abnormal muscle patterns were substantiated by longer duration of extensor muscle contraction and lower amplitude of flexor muscle contraction. In the fMRI study, abnormal activation patterns were seen in the prefrontal-premotor-parietal network (***chapter 2***). Both studies therefore strongly suggest that CD is not a focal, but rather a generalized movement disorder. Although clinically normal, the other non-dystonic body parts may already be affected. We introduced this concept as ***pre-dystonic state***. This concept is consistent with previous descriptions of endophenotypic traits in focal and task-specific dystonia. In writer's cramp patients, the representation of the hand in S1 showed anatomical and functional abnormalities in the dystonic and non-dystonic hemispheres. The authors proposed that degradation of the somatotopic maps of the hands in S1 can be considered as an endophenotype (Meunier et al., 2001). In focal dystonia, illusion of movement was impaired after fatigue not only in patients but also in their first-degree relatives (Frima et al., 2008).

The hereditary susceptibility of endophenotypic traits may indicate that a pre-dystonic state is caused by genetic influences. Genetic factors have been established in generalized dystonia and presumed to be present in focal and task-specific dystonia. The frequency of positive family members is higher than expected by chance in focal dystonia (Horstink et al., 2007) while dystonic

endophenotypic traits have been described in relatives of CD patients (Frima et al., 2008). This raises the question if generalized dystonia is similar to focal and task-specific dystonia. Degradation and particularly dedifferentiation of somatosensory neurons have also been noted in generalized dystonia. During placement of electrodes for deep brain stimulation, a loss of selectivity of “somatosensory” neurons in the globus pallidus and thalamus were exhibited (Lenz et al., 1999). Structural abnormalities of volume and water diffusion in basal ganglia and cortex were found by others in generalized, focal and task specific dystonia (Draganski et al., 2003; Etgen et al., 2006; Garraux et al., 2004) and also in our own patient group (***chapter 6***). Our study particularly showed gray matter abnormalities in magnitude and directionality of molecular diffusion in right thalamus, bilateral globus pallidus and putamen, and left caudate, determined with voxel-wise analysis The gray matter volume, measured with voxel-based morphometry, was also abnormal in the same regions in a DTI study studying CD and blepharospasm patients (Obermann et al., 2007). Cortical diffusivity abnormalities were seen in our study in the left precentral gyrus. M1/S1 structural volume deviations were observed in other generalized, task-specific and focal dystonias as well (Draganski et al., 2003; Garraux et al., 2004), with additional structural volume abnormalities in the cerebellum (Delmaire et al., 2007) and left inferior parietal cortex (Egger et al., 2007; Etgen et al., 2006). White matter water flow disruption was demonstrated in our patient group in the vicinity of right thalamus and middle frontal cortex, and bilaterally near the putamen and caudate nucleus. These results are in concordance with DTI studies using voxel-wise analysis in CD (Colosimo et al., 2005) and blepharospasm (Fabbrini et al., 2008). Although genetic factors and structural abnormalities seem to be a uniform finding in the different forms of dystonia, it does not explain the differences between age of onset and distribution of dystonia in the focal and task-specific forms compared to the generalized form. Additionally, patients with focal and task-specific dystonia do not seem to progress into generalized dystonia. Thus, one etiological factor for these dystonia entities can not be pointed out directly. They may comprise a continuum between environmental and genetic factors (Meunier et al., 2001).

Even though a genetic susceptibility may be an etiological component in a pre-dystonic state in the body, environmental triggers are frequently present before the onset of focal dystonia. In clinical practice, patients sometimes report a neck or limb injury prior to the symptoms (Jankovic, 2001), and patients with complex regional pain syndrome may develop dystonia (Gieteling et al., 2008). Animal studies found that this change in sensory input from a limb to the brain due to an injury may indeed trigger focal dystonia. Rat models were used to induce blepharospasm. The researchers found that only the rats with a combination of dopamine level depletion (i.e. basal ganglia dysfunction) and orbicularis oculi weakening developed focal dystonia, while depletion of dopamine or muscle weakening alone did not result in the disease (Schicatano et al., 1997). Also, overactivity of a body part as for example in musicians or writers may act as such a trigger. This is confirmed by monkey studies showing that repetition of stereotyped movements in hand or neck provokes a specific task-induced dystonia (Byl et al., 1996; Evinger, 2005).

The proposed concept of a pre-dystonic state may have direct clinical implications. Approximately 20% of patients with CD develop dystonia in other parts of the body resulting in segmental dystonia (Dauer et al., 1998). By recording EMG from clinically non-dystonic parts of the body at regular intervals, the progression of a focal dystonia into segmental dystonia may be acknowledged early, predicted, or may be prevented. In our study, the mean extensor burst duration during wrist flexion/ extension seems to have most predictive power for future longitudinal studies. The sensitivity and specificity of this measure in CD are relatively high (62.5% and 75% respectively) and variations in this measure due to electrode placement variability are smaller compared to EMG amplitude measurement. However, one has to keep in mind that a prediction of a spread of dystonia into another body part may not be relevant for a patient if a proper treatment can not be provided.

A dystonia model

We propose that focal dystonia is a movement ***preparation*** disorder resulting from a complex interplay of basal ganglia dysfunction, altered processing of

sensory input from a specific body part to the cortex and impaired sensorimotor integration. The basal ganglia modulate sensorimotor circuits by selective inhibition within striato-cortical networks. When the basal ganglia do not function properly, dopamine levels may decrease and the inhibitory effect on the striato-cortical networks may be reduced. The cortical areas then become more easily excited by a low-threshold input. Structural abnormalities have been identified in especially putamen but also caudate nucleus and globus pallidus in patients with CD (***chapter 6***), focal hand dystonia (Black et al., 1998) and blepharospasm (Etgen et al., 2006). Although basal ganglia dysfunction does not explain why one body part is affected with dystonia while another is not, it could explain why some subjects are more susceptible to dystonia. Impaired basal ganglia function may affect the whole body and direct all body parts into a pre-dystonic state. This is supported by our and other neuroimaging studies since movements in dystonic body parts, such as writing in writer's cramp (Ibanez et al., 1999; Preibisch et al., 2001), as well as movements in pre-dystonic body parts (hand movement in CD; ***chapter 2***), are related with abnormal basal ganglia activation. Changes in cortical activation were also observed in these studies, suggesting that the cortex adapts to the changed basal ganglia output. With PET, functional changes in the cortex have found as a result of dynamic hand immobilisation after tendon surgery. During immobilization, impaired striatal activation has been found together with increased activation in parietal and cingulate cortices, clinically resulting in a temporarily clumsy hand. When the movement ability was restored after 6-8 weeks, normal striatal activation was found again together with a decrease in cingulate activation (de Jong et al., 2003). Beyond their initial compensatory attempt, one might thus reason that the cortical regions helped to reset the striatum to perform "automated" movements again.

Nevertheless, an additional factor seems to be necessary to push a pre-dystonic body part into a dystonic state. This factor has to contain a mechanism that reduces parietal and premotor activation so that the striatum cannot be reset due to insufficient compensation. Abnormal sensory input can bring about abnormal parietal activation (Tinazzi, 2003). Sensory input can change after a minor but painful accident (Jankovic, 2001) or in relation to stereotyped repetition of

movement. Patients frequently report sensory phenomena preceding the onset of dystonic symptoms. Also, alleviation of dystonic symptoms after overt and imagined sensory tricks supports the hypothesis of sensory input modification (Schramm et al., 2004; Schramm et al., 2007). Still, a normal brain should be able to adapt to a reduced sensory input by increasing the "set-point" for which it adequately excites the sensorimotor pathway. When basal ganglia dysfunction reduces the inhibitory effect on the striato-cortical network, the adaptive process initiated by changes in sensory input may either lose the set-point or increase it continually. Then, movement performances are associated with skewed movements.

One final consideration needs to be made in respect to our proposed (pre)dystonia model. We propose that the prefrontal activation increases are due to cerebral adaptive mechanisms (***chapter 5***), while other studies in task-specific and idiopathic torsion dystonia rate the prefrontal cortex increases as a dystonic phenomenon (Ceballos-Baumann et al., 1995; Ceballos-Baumann et al., 1997; Playford et al., 1998). The same can be said about basal ganglia activation. Our study shows underactivation in the striatum (***chapter 2***) while others report striatal hyperactivation during movement in a dystonic body part (Ceballos-Baumann and Brooks, 1997). We speculate that reduced basal ganglia activation is a finding in the pre-dystonic state, which can still be compensated by cortical regions as prefrontal and posterior parietal cortex. This is the reason that the patient still has clinically normal hand movements. However, disease progression may further intensify the cortical drive, now originating from widespread cortical regions, to the targets in the striatum. Over time, this overstimulation of more cortical fields that are less function-specific may result in a reduced selection capacity within the basal ganglia. The loss of the latter may demarcate the transition from pre-dystonic into a dystonic state. As normal selection within the basal ganglia is thought to be based on surround inhibition (Mink, 1996), loss of this inhibition leads to overactivation within the basal ganglia and subsequent loss of inhibition on especially prefrontal areas, since the striatum mainly projects to this part of the cortex (Lehericy et al., 2004).

Future perspectives

In the future, specific studies on pathophysiological, diagnostic and therapeutic levels are recommended. To improve understanding of the pathophysiology of dystonia, determination of (ab)normal sensory input during pre-dystonic body part movement may be suggested, for example using muscle vibration. Also, cerebral activation patterns during pre-dystonic body part movement in other focal dystonias may be interesting to identify. For the diagnostic part, a longitudinal study with EMG could be relevant to investigate a spread of focal to segmental dystonia and to predict this spread by measuring extensor muscles during a clinically normal movement of a body part adjacent to the dystonic body part. To extend the therapeutic options, TMS on the left superior parietal cortex may be a promising tool, although our choice of this region needs to be evaluated further. Also, ***stimulation*** above 1 Hz instead of ***inhibition*** at 1 Hz of the parietal cortex may "normalize" cerebral activation patterns and improve dystonic symptoms.

Chapter 9

Nederlandse samenvatting

Nederlandse samenvatting

Dystonie is een bewegingsstoornis waarbij spieren niet goed samenwerken als gevolg van een verkeerde aansturing van deze spieren door de hersenen. Daardoor ontstaan draaiende, wringende bewegingen en abnormale houdingen van een bepaald lichaamsdeel (een zogenaamd dystoon lichaamsdeel). Er zijn verschillende vormen van dystonie. In dit onderzoek is specifiek gekeken naar cervicale dystonie (CD), waarbij alleen de spieren in de nek dystoon zijn. Naar schatting hebben ongeveer 3000 mensen in Nederland CD. De oorzaak van CD is nog niet bekend. Genetische factoren lijken een belangrijke rol te spelen, omdat een kwart van de CD patiënten een eerstegraads familielid heeft met een vorm van dystonie. Ook omgevingsfactoren lijken erbij te zijn betrokken. Een deel van de patiënten heeft CD ontwikkeld na nekletsel door een trauma of door het te vaak uitvoeren van dezelfde beweging.

De afgelopen jaren is er uit wetenschappelijke onderzoek gebleken dat bewegingen van een dystoon lichaamsdeel overeenkomen met veranderde activiteit in hersengebieden die betrokken zijn bij voorbereiding en uitvoer van een beweging. Een normale beweging wordt voorbereid in de pariëtale schors en de prefrontale schors. In de pariëtale schors komt informatie van zintuigen binnen die wordt gebruikt om een beweging voor te bereiden. In de prefrontale schors wordt het doel en het effect van de geplande beweging vastgesteld. Deze informatie wordt gecombineerd in de premotore schors en overgebracht naar de primaire motor schors. Dit laatste gebied stuurt de spieren aan die nodig zijn voor de uitvoer van de beweging. De bewegingsuitvoer wordt gecontroleerd door de basale ganglia, die bepaalde bewegingen versoepelen en andere onderdrukken. Bij patiënten met andere vormen van dystonie dan CD blijkt dat niet alleen de hersenactiviteit tijdens de uitvoer van de beweging (primaire motor schors, basale ganglia) is veranderd, maar dat al tijdens de voorbereiding van beweging (prefrontale, premotore en pariëtale schors) veranderde hersenactiviteit is te zien. Deze hersenactiviteit kan tijdelijk worden “genormaliseerd” bij CD door extra zintuiginformatie naar de pariëtale schors te sturen (bijv. via aanraking van de kin). Daarnaast blijkt deze afwijkende hersenactiviteit niet alleen meetbaar tijdens het

uitvoeren van een taak met een dystoon lichaamsdeel, maar ook bij taakuitvoering met een uiterlijk normaal, niet-dystoon lichaamsdeel. Dit zou betekenen dat dystonie niet één lichaamsdeel aantast, maar het hele lichaam beïnvloedt.

Onderzoek naar beweging wordt vaak gedaan met functionele magnetische resonantie imaging (fMRI). Hierbij voert een proefpersoon een taak uit in de MRI scanner terwijl de scanner direct de activiteit in de hersengebieden meet. Bij patiënten met bewegingsstoornissen als CD wordt de hersenactiviteit vergeleken met gezonde vrijwilligers. Hierdoor kan een veranderde activiteit in bepaalde gebieden worden aangetoond tijdens het uitvoeren van die specifieke taak. Ook is het mogelijk om de activiteit in hersengebieden van buitenaf te beïnvloeden met transcraniële magnetische stimulatie (TMS). De TMS geeft magnetische pulsen af die in de hersenen worden omgezet in een elektrisch stroompje. Dit kan de hersenactiviteit verlagen of verhogen. Het is mogelijk om TMS toe te passen in een MRI scanner. Het effect van TMS (activiteitsverandering in een bepaald hersengebied) kan direct worden gemeten met fMRI door de hersenactiviteit te meten tijdens een taak voor en na TMS. Ook kan worden bekeken wat voor effect een verandering van activiteit in één hersengebied heeft op de activiteit van andere hersengebieden.

Dit promotieonderzoek had tot doel om:

(I) abnormale hersenactiviteit bij CD in kaart te brengen met fMRI met specifieke aandacht voor de pariëtale schors,

(II) deze hersenactiviteit in de pariëtale schors te beïnvloeden met TMS,

(III) abnormale hersenactiviteit in verband te brengen met abnormale aanspanning van spieren,

(IV) anatomische afwijkingen in de hersenen aan te tonen die abnormale hersenactiviteit zouden kunnen verklaren.

Het eerste onderzoek (***hoofdstuk 2***) was gericht op identificatie van abnormale hersenactiviteit met fMRI bij acht CD patiënten in vergelijking met negen gezonde vrijwilligers. De taak was een beweging van een niet-dystoon lichaamsdeel, namelijk de rechterpols. Hiermee wilden we bekijken of niet-dystone lichaamsdelen ook afwijkingen in hersenactiviteit lieten zien. Het buigen en strekken van de

rechterpols werd uitgevoerd én verbeeld. Proefpersonen bedachten dat ze de beweging maakten zonder dat deze ook daadwerkelijk werd uitgevoerd. Verbeelding van beweging activeert hersengebieden die belangrijk zijn voor de voorbereiding van beweging zonder dat hersengebieden voor de uitvoer van beweging zijn betrokken. Hierdoor kon worden gekeken naar het gebruik van zintuiginformatie bij het voorbereiden van een beweging zonder dat zintuiginformatie tijdens de uitvoer van beweging de hersenactiviteit vertroebelde. Uit de resultaten bleek dat tijdens het verbeelden van beweging CD patiënten lagere activiteit lieten zien in de pariëtale schors en premotore schors. Het uitvoeren van de beweging liet tevens een lagere activiteit zien in de basale ganglia bij patiënten. CD patiënten lieten bij verbeelding en uitvoer van beweging hogere activiteit zien in de prefrontale schors. Op basis van deze resultaten en de anatomische connecties tussen de verschillende hersengebieden werd vermoed dat de pariëtale schors bij CD minder goed functioneerde waardoor de premotore schors ook minder goed kon functioneren. CD patiënten probeerden dit te compenseren door de activiteit in de prefrontale schors te laten toenemen.

Om dit vermoeden te testen werd het tweede onderzoek opgezet met gebruik van TMS in de MRI scanner. Met TMS werd de activiteit in de linker pariëtale schors verstoord bij tien gezonde vrijwilligers en zeven CD patiënten. Dit gebied was gekozen, omdat hier in het eerste onderzoek een lagere activiteit was te zien bij CD patiënten. TMS boven dit hersengebied bij gezonde vrijwilligers liet vergelijkbare veranderingen in hersenactiviteit zien als bij CD patiënten waren ontdekt zonder TMS. Dit bestond uit verhoogde activiteit in de prefrontale schors en verlaagde activiteit in de premotore en pariëtale schors (***hoofdstuk 4***). Verstoring van de activiteit in de pariëtale schors door TMS bij CD patiënten gaf een verdere verlaging van de al lage activiteit in de pariëtale schors en een verdere verhoging van de al hoge activiteit in de prefrontale schors, vooral tijdens verbeelden van de beweging (***hoofdstuk 5***). Op basis van de resultaten van TMS en fMRI concludeerden we dat de abnormale hersenactiviteit bij CD patiënten inderdaad kon ontstaan door een verminderde activiteit van de pariëtale schors. Dit zou betekenen dat het voorbereiden van een beweging (en het gebruik van zintuiginformatie) al is aangetast en niet alleen de uitvoer van een beweging. Het is

opvallend dat de verlaagde hersenactiviteit werd gecompenseerd door verhoogde activiteit in de prefrontale schors. Dit leek ervoor te zorgen dat een niet-dystoon lichaamsdeel een uiterlijk normale beweging kon uitvoeren ondanks abnormale hersenactiviteit.

Dit bracht ons naar de volgende onderzoeksvraag: Zorgt abnormale activiteit in bewegingsgerelateerde hersengebieden voor abnormale aanspanning van spieren terwijl de beweging zo te zien normaal wordt uitgevoerd? Om deze vraag te beantwoorden lieten we acht gezonde vrijwilligers en acht CD patiënten dezelfde beweging maken met de rechterpols terwijl de spieractiviteit werd gemeten (***hoofdstuk 3***). Er bleken inderdaad verschillen te zijn in de spieractiviteit tussen beide groepen terwijl de polsbeweging bij alle proefpersonen goed werd uitgevoerd. CD patiënten hadden een gemiddeld lagere spieraanspanning bij buigen van de pols, terwijl strekken van de pols zorgde voor een gemiddeld langere spieraanspanning. Daarnaast was er grotere variatie te zien bij het bewegen van de pols bij CD patiënten ten opzichte van gezonde vrijwilligers. Het lijkt er dus op dat abnormale hersenactiviteit ondanks compensatiemechanismen in de hersenen wel zorgt voor abnormale spieraanspanning die niet direct is waar te nemen.

In het laatste deel van dit promotieonderzoek werd gekeken naar de aanwezigheid van anatomische afwijkingen die de abnormale hersenactiviteit zouden kunnen verklaren (***hoofdstuk 6***). Hiervoor werd gebruikt gemaakt van een nieuwe scantechniek (diffusion tensor imaging) wat is gebaseerd op het feit dat moleculen vrij langs een zenuwvezel bewegen, maar worden gehinderd in hun bewegingen bij kruisingen van vezels. Door de bewegingsrichting van watermoleculen te meten kan daardoor indirect de richting van de zenuwvezels zichtbaar worden gemaakt. Door een anatomische kaart met zenuwvezels van CD patiënten te vergelijken met die van gezonde vrijwilligers werd ontdekt dat CD patiënten een andere zenuwvezel oriëntatie hebben in de basale ganglia. Dit zou een verklaring kunnen zijn voor de abnormale hersenactiviteit tijdens de uitvoer van beweging. Er werden echter geen anatomische afwijkingen gevonden in de pariëtale schors, dus abnormale activiteit tijdens voorbereiding van beweging kon niet door anatomische afwijkingen in dit gebied worden verklaard.

De resultaten van dit promotieonderzoek hebben geleid tot het opstellen van een model voor het ontstaan van dystonie. Mogelijk hebben CD patiënten een genetische aanleg om anatomische afwijkingen in de basale ganglia te ontwikkelen. Een afwijkende oriëntatie van zenuwvezels zou kunnen zorgen voor een verandering van de activiteit in de hersenschors. Abnormale hersenactiviteit beïnvloedt de spieraanspanning in het hele lichaam. Uiterlijk hoeft een beweging echter niet aangedaan te zijn door effectieve compensatiemechanismen in de hersenschors. We noemen deze fase van de bewegingsstoornis pre-dystonie. De overgang van pre-dystonie naar dystonie van bijvoorbeeld de nek zoals bij CD zou kunnen worden uitgelokt door een stoornis in de zintuiginformatie. Aangezien extra zintuiginformatie naar de pariëtale schors CD tijdelijk kan verbeteren, is het ook mogelijk dat een trauma of overmatig gebruik van de nek leidt tot minder zintuiginformatie. Hierdoor zou de hersenactiviteit in de pariëtale schors zodanig kunnen veranderen dat een persoon CD ontwikkelt.

Chapter 10

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Chapter 11

Dankwoord

Dankwoord

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Chapter 12

List of Publications

List of publications

Alffenaar, J.W., de Vries, P.M., Luijckx, G.J., van Soolingen, D., van der Werf, T.S., van Altena, R., 2008. Plasma and cerebrospinal fluid pharmacokinetics of moxifloxacin in a patient with tuberculous meningitis. Antimicrob. Agents Chemother. 52, 2293-2295.

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