Investigating the role of the cerebellum in idiopathic focal dystonia

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Colofon

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by

Britt Sofie Hoffland

born on February 11, 1987
in Amsterdam

Voor mijn ouders

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Appendices

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Introduction

Dystonia

Dystonia is one of the so-called hyperkinetic movement disorders: a group of involuntary movements that occur in excess of willed movements (other members of this group include chorea, tremor and tics). Dystonia as a movement disorder was recognized first by Schwalbe in a Jewish Eastern European family in 1908 [1]. The term dystonia was, however, first used by Oppenheim in 1911 [2]. Today, the estimated prevalence of this neurological disorder is 15-30 / 100,000, increasing to more than 700/100,000 above the age of 50 years [3].

Dystonia is characterized by sustained or intermittent muscle contractions causing abnormal, often repetitive movements, abnormal postures, or both. Dystonic movements are typically patterned, twisting and may be tremulous. Dystonia is often initiated or exacerbated by voluntary action, either in the affected body part itself, but also by movements in other body parts [4]. Some forms of dystonia are task specific, occurring only when performing a certain activity, such as writing (writer's cramp) or playing the piano (musician's dystonia).

There are other abnormal motor phenomena that can be observed in patients with dystonia. Dystonic tremor can coexist, which is a rhythmical, patterned movement produced by contractions of dystonic muscles that is frequently aggravated by an attempt to maintain normal posture. Performing a (dystonia-provoking) movement with a non- affected limb can induce so-called mirror movements: dystonia in the opposite, affected limb. "Sensory tricks" describe the observation that dystonic postures can sometimes be improved temporarily by simply touching part of the body (without using force), mostly by the patient themselves [4].

Dystonia is currently classified along two axes: clinical characteristics and aetiology. Clinical characteristics are for example age at onset, body distribution, temporal pattern and associated features. Dystonia can coexist with another movement disorder or other neurological or systemic symptoms. Regarding aetiology, dystonia can be inherited (proven genetic origin) or acquired (for example due to cerebral infarction or drugs). Dystonia may also be idiopathic, i.e. of unknown cause. This implies that there is no direct proven/ suspected genetic origin, evident structural brain lesion, or degenerative brain disease [4].

Cervical dystonia, blepharospasm and writer's cramp are the most common forms of adult-onset focal dystonia and are most often of idiopathic origin. Cervical dystonia is characterized by abnormal head, neck and shoulder positions due to dystonic contractions of cervical muscles. In blepharospasm, the muscles around the eyes are affected (most often orbicularis oculi muscles) resulting in involuntary eye lid closure. Writer's cramp is a task-specific form of hand dystonia in which writing induces involuntary excessive muscle contractions. These three conditions are referred to as idiopathic focal dystonias, or formerly primary focal dystonias. In this thesis, the focus will be on these three types of dystonia.

Although there is no overt brain lesion in idiopathic focal dystonia, some neuro-imaging studies have revealed subtle abnormalities in several brain regions (including the cerebellum) [5]. Also, a familial occurrence of focal dystonia occurs in about 25 percent of cases, indicating a genetic background [6]. So, a deeper understanding of the aetiology of idiopathic focal dystonias, and perhaps reclassification, will very likely be required in the future.

Reduced mobility, pain and psychosocial concerns make dystonia a disabling movement disorder. The current treatment of most forms of dystonia is symptomatic, which is mainly due to the fact that the exact pathophysiology of dystonia remains elusive. The basal ganglia are central in motor control and were traditionally considered to be key players in the pathophysiology of dystonia. However, accumulating evidence from various sources currently points to an additional role of the cerebellum, although the exact contribution of the cerebellum to the pathophysiological processes in dystonia is far from clear [5].

The aim of this thesis is to further unravel the role of the cerebellum in the pathophysiology of idiopathic, focal dystonia. We have used several neuroscientific tools to address this issue and these tools are briefly described below.

Box 1 | TMS

Transcranial magnetic stimulation (TMS) is a non-invasive method of stimulating brain neurons. A magnetic field generator sends a current through an electromagnetic coil that is held over the subject's head. The current creates a magnetic field and induces weak electric currents in nearby brain tissue. When the coil is held over the motor cortex and sufficient intensity of stimulation is used, the corticospinal pathway will be activated, resulting in the activation of corresponding muscles that can be recorded by surface electromyography (EMG). This motor response is called the motor evoked potential (MEP).

The period of electrical silence in the surface EMG directly after the MEP when the stimulus is delivered during tonic muscle contraction is called the cortical silent period (CSP). It is thought to be caused by both spinal and cortical inhibitory mechanisms, but given the short duration of the former, the total duration of the CSP is usually only altered by cortical mechanisms.

Several TMS applications have been developed to investigate the physiology of the motor system. In chapter 3.1, we assessed the excitability of intracortical inhibitory and excitatory motor cortex circuits at rest using a paired pulse protocol. We assessed short interval intracortical inhibition (SICI). In SICI a subthreshold stimulus, applied at an interstimulus interval of 1-6 ms before a test stimulus, reduces the motor evoked

potential size compared to those produced by the test stimulus alone. SICI is a complex measure and likely reflects a balance between cortical inhibition and facilitation. Given that drugs that enhance GABAa- ergic neurotransmission also increase SICI, SICI has been proposed to reflect post-synaptic cortical inhibition mediated by this neurotransmitter. We also investigated intracortical facilitation (ICF), the methods to assess ICF are similar to that of SICI but a longer interstimulus interval of 6-30 ms is used to cause enhancement of the motor evoked potential size of the test stimulus. The physiological basis of ICF is less well understood, excitatory glutamergic circuits in the motor cortex have been proposed to be involved.

In chapter 3.2, we used TMS to study cerebellar brain inhibition (CBI), an inhibitory circuit which is thought to be mediated through the dentato-thalamo-cortical pathway. In CBI, the size of the motor evoked potential elicited by a TMS pulse over one hand motor area is significantly reduced by a TMS pulse 5-7 ms earlier, delivered over the contralateral cerebellar hemisphere. It is believed that activation of cerebellar Purkinje cells inhihibits M1 via a disynaptic pathway including the deep cerebellar nuclei and the ventro-lateral thalamus.

In chapter 4, we used repetitive TMS. This is a different form of TMS that, depending on the stimulation characteristics, can either increase or decrease the excitability of the stimulated brain region and its connected pathways. Importantly, these changes in excitability can persist for a brief period after the actual stimulation has stopped. It is widely believed to be caused by changes in synaptic efficacy similar to long-term potentiation (LTP) and long-term depression (LTD). We used one specific form of repetitive TMS, namely continuous Theta Burst Stimulation (cTBS), to induce inhibitory cortical after-effects in the stimulated cerebellar cortex, thereby transiently disrupting cerebellar functioning [7].

Box 2 | Eyeblink classical conditioning

Eyeblink classical conditioning (EBCC) is a form of associative motor learning in which paired presentation of a conditioned (CS; auditory tone) and unconditioned stimulus (US; electrical stimulus or air puff) results in a conditioned eyeblink response (CR) (see figure 1). The cerebellar cortex, cerebellar nuclei, and inferior olives are critical neuroanatomical sites for the acquisition and retention of CRs [8-10]. Therefore, abnormal EBCC is a neurophysiological indicator of cerebellar dysfunction. In chapter 5, we applied cTBS (continuous Theta Burst Stimulation) over the right cerebellar hemisphere and measured its after-effects on acquisition and retention of EBCC in patients with idiopathic cervical dystonia and healthy controls.
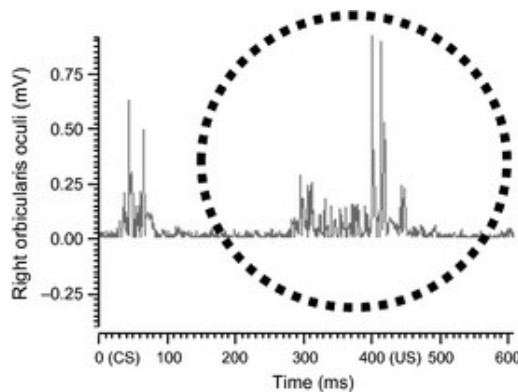
Figure 1 | Screenshot of EMG recording over the right orbicularis oculi (OO) muscle during EBCC. The CS (auditory tone) produces an acoustic startle response (alpha blink). A conditioned response (CR) is visible before the onset of the US (supraorbital nerve stimulus).

Box 3 | Vicon & Split-belt treadmill

Vicon is a motion capture system. Using reflective markers on anatomical landmarks, three- dimensional kinematics are recorded using a 6-camera motion analysis system (see figure 2). Marker position data are subsequently analysed offline using custom written software in Matlab (MathWorks, Natick, USA) to investigate gait parameters. In chapter 6, we studied gait parameters during split-belt walking to investigate sensorimotor adaptation. We used a split-belt treadmill, consisting of two separate belts with independently controllable speeds (ForceLink BV, The Netherlands) in combination with the Vicon Plug-in-Gait model (Vicon Motion Systems, Oxford, UK).

During this task, participants are asked to adjust to a new type of walking pattern on a treadmill with various speeds for each leg. Two types of gait adjustments are seen during split-belt walking: 1) direct reactive adjustments of walking parameters (e.g. stride length and time in stance) to accommodate the novel difference in belt speeds, and 2) more gradual adaptive feedforward adjustments in step length, time in double support, oscillation and phasing parameters that persist as aftereffects when the perturbation is removed. Direct reactive adjustments are thought to rely predominantly on spinal circuitry. The modification of motor programs through trial-and-error practice that is seen during sensorimotor adaptation is believed to be strongly dependent on cerebellar functioning.
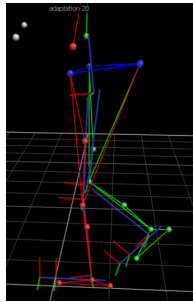
Figure 2 | Screenshot of Vicon motion capture system recording.

Box 4 | fMRI

Functional Magnetic Resonance Imaging (fMRI) is a neuroimaging technique to investigate brain activity during a certain task. It records the haemodynamic response (changes in blood flow) by measuring the level of deoxygenated haemoglobin in the blood, i.e. the blood-oxygen-level dependent (BOLD) signal. fRMI does therefore not measure neuronal activity directly, but indirectly as cerebral flood flow and neuronal activation are coupled [11].

The cerebral activity pattern during a task is calculated by investigating the correlation between the BOLD signal and a behavioural parameter, for example pressing a button. During our fMRI experiment in chapter 7, we asked our subjects to perform two motor learning tasks with different basal ganglia and cerebellar contributions. These two motor learning tasks were kept as similar as possible, because when they would differ in more than one way, there could be multiple explanations for observed differences in cerebral activity.
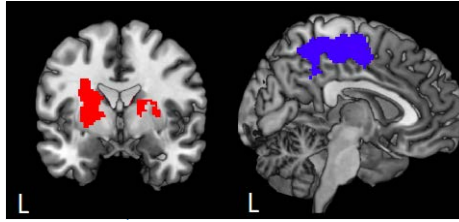
Figure 3 | Image of the brain with statistically significant areas between patients and controls during a visuomotor learning task. L indicates left side of the brain.

Outline of this thesis

In **chapter 2**, I summarize and review the existing literature, that was available at the start of this research project, which point to a cerebellar role in the pathophysiology of dystonia. We evaluate knowledge provided by numerous sources: 1) work detailing cerebellar connectivity in primates, 2) data that suggests a role for the cerebellum in the generation of dystonia in rodent models, and 3) clinical observations, imaging studies and electrophysiological findings in patients with dystonia and in patients with structural lesions and heredodegenerative disorders of the cerebellum. After this, new studies investigating the role of the cerebellum in dystonia have been done (including the ones described in this thesis), and I will discuss the meaning and interpretation of these more recent findings in the final chapter of this thesis.

An early inspiration to start suspecting a role of the cerebellum in dystonia, is the observation of patients with the so-called syndrome of dystonia and cerebellar ataxia (DYTCA). They have clinically mild and slowly progressive cerebellar ataxia, but the concomitant dystonia is more prominent and disabling [12, 13]. In **chapter 3.1**, we use transcranial magnetic stimulation (TMS) to investigate the cortical excitability profile of patients with this rare syndrome. The dystonia in DYTCA might be due to mechanisms that are different from those in idiopathic dystonia. More specifically, we wonder whether an abnormal cortical excitability profile, the typical fingerprint in idiopathic dystonia, is also an important driver for dystonia in DYTCA.

As a next step, we further investigate the role of the cerebellum in the regulation of cortical excitability in healthy controls in **chapter 3.2**. We test whether there is a cerebellar contribution to surround inhibition (SI), which is the muscle-specific modification of the excitability of the corticospinal pathway. SI is aberrant in dystonia [14]. Cerebellar brain inhibition (CBI) is an inhibitory circuit which is thought to be mediated through the dentato-thalamo-cortical pathway [15, 16]. Using transcranial magnetic stimulation (TMS), we examin the relationship between SI and CBI.

One of the first actual indicators of abnormal cerebellar functioning in idiopathic dystonia is the impairment of eyeblink classical conditioning (EBCC), a cerebellum-dependent paradigm of associative motor learning [8-10]. In **chapter 4**, we first try to reproduce previous findings of EBCC deficits in idiopathic cervical dystonia, but also aim to investigate whether these can be modified by practice (via repeated sessions of EBCC) and by direct non-invasive modulation of cerebellar excitability (through inhibitory continuous theta burst stimulation) [17]. We do this to answer the question of whether impaired EBCC in patients with idiopathic focal dystonia reflects actual cerebellar pathology, or is due to a more functional cerebellar defect. As a last EBCC experiment, we use this tool to study cerebellar learning in patients with the so-called fixed dystonia syndrome, examining whether the EBCC pattern is similar to that in typical, organic dystonia.

In **chapter 5,** we explore whether abnormalities of cerebellar motor learning in idiopathic focal dystonia are solely detectable in more pure forms of cerebellum-dependent associative motor learning paradigms, such as EBCC, or whether these are also present in other motor learning paradigms that rely heavily on the cerebellum, but that additionally require a more widespread, intact cerebral sensorimotor network. For this purpose, we choose a motor learning gait paradigm on a split-belt treadmill [18]. We obtain and analyze kinematic data recorded by a motion analysis system (Vicon Motion Systems, Oxford, UK) of both healthy controls and patients with different forms of idiopathic focal dystonia, to investigate whether possible abnormalities are convergent or divergent for the different dystonia subtypes.

Previous studies suggest that the cerebellum might be acting as a compensatory mechanism in dystonia [5]. In our last study, we test this hypothesis in an fMRI set-up that directly targets both cerebellar and basal ganglia functioning in **chapter 6**. We investigate two forms of motor learning, visuomotor and sequence learning, known to rely predominantly on the cortico-striatal and the cortico-cerebellar loops, respectively, to explore how the cerebellum and basal ganglia interact in this movement disorder [19-22].

In **chapter 7** I provide separate summaries of these studies, trying to crosslink our findings with the available literature and current hypotheses, and provide new research directions. I end with an integrated discussion of the role of the cerebellum in dystonia. A short Dutch summary is also included in this chapter.

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The cerebellum in dystonia
– Help or hindrance?

Abstract

Dystonia has historically been considered a disorder of the basal ganglia. This review aims to critically examine the evidence for a role of the cerebellum in the pathophysiology of dystonia. We compare and attempt to link the information available from both clinical and experimental studies; work detailing cerebellar connectivity in primates; data that suggests a role for the cerebellum in the genesis of dystonia in murine models; clinical observation in humans with structural lesions and heredodegenerative disorders of the cerebellum; and imaging studies of patients with dystonia. The typical electrophysiological findings in dystonia are the converse to those found in cerebellar lesions. However, certain subtypes of dystonia mirror cerebellar patterns of increased cortical inhibition. Furthermore, altered cerebellar function can be demonstrated in adult onset focal dystonia with impaired cerebellar inhibition of motor cortex and abnormal eyeblink classical conditioning. We propose that abnormal, likely compensatory activity of the cerebellum is an important factor within pathophysiological models of dystonia. Work in this exciting area has only just begun but it is likely that the cerebellum will have a key place within future models of dystonia.

Introduction

Dystonia has long been considered to be a manifestation of basal ganglia dysfunction, similar to other movement disorders. However, there is accumulating evidence from a wide variety of sources that the cerebellum may have a role to play in the pathophysiology of dystonia. Here we review this evidence, and demonstrate how the intimate structural and functional connections between cerebellum and basal ganglia appear to be involved in patients with dystonia.

Anatomy

The cerebellum and the basal ganglia receive input from multiple cortical areas and have been traditionally been thought to modulate motor control via distinct thalamic nuclei that project to the primary motor cortex [1]. However studies using viral tracers in primates reveal the macro-architecture of an increasing number of cerebellar and basal ganglia projections. Multisynaptic circuits link the cerebellum and basal ganglia with the primary motor cortex, supplementary motor area (SMA), pre-SMA, oculomotor, prefrontal, and posterior parietal cortex [2-5]. Many cortical areas project topographically to specific cerebellar and basal ganglia territories that reciprocally innervate these same cortical areas[6]. There is also a substantial direct communication between the basal ganglia and the cerebellum: a disynaptic projection linking the dentate nucleus (output stage of cerebellar processing) to the striatum (input stage of basal ganglia processing)[7], and a forward connection from the subthalamic nucleus of the basal ganglia to the cerebellar cortex [8]. The reciprocal communication between these two major subcortical structures suggests that they directly modulate each other. Brainstem nuclei provide another junction for the cerebellum and basal ganglia to interact, for example in cats the red nucleus receives input from both the basal ganglia and cerebellar nuclei which then project directly to motor nuclei [9]. These neuronal circuits provide an anatomical substrate for the cerebellum and basal ganglia to have wide ranging functions in motor and non-motor domains. Dysfunction in either structure could induce either compensatory activity or disruption in the other.

To date, there is a paucity of information regarding the neuropathology of dystonia, and there has not been specific exploration of cerebellar pathology or cerebellar-basal ganglia projections in brains of dystonia patients. Reported autopsy studies in sporadic primary dystonia and DYT 1 dystonia have not shown an overt neurodegenerative process or clear patterns of cell loss [10, 11].

Animal models

Pharmacological and mutant mouse models of dystonia provide further data supporting a role of the cerebellum in the genesis of dystonia[12, 13]. For example, tottering mice mutants exhibit paroxysmal dystonia due to a point mutation in a gene that codes for a calcium channel [14]. Clinically and electrophysiologically these episodes have characteristics similar to human dystonia[15]. Surgical removal of the cerebellum abolishes dystonic attacks in these

mice[12]. Elimination of dystonic movements following cerebellectomy has also been found in other murine models of dystonia [16, 17]. Similarly, dystonia is abolished if the tottering mouse is bred with an additional genetic mutation that causes Purkinje cell degeneration [18]. In a pharmacological mouse model for dystonia, microinjection of low doses of kainic acid into the cerebellar vermis of mice generates dystonia of a severity proportional to kainite dose [19]. Microdialysis of the striatum reveals dystonic attacks to be associated with reductions in striatal dopamine in both tottering mice and the kainic acid pharmacological model, which suggests that that cerebellar activity can directly influence the dynamics of striatal dopamine [12]. Recently, a pharmacological model of rapid onset dystonia parkinsonism (DYT12) has been created by selective blockade of the sodium–potassium ATPase pump (which is mutated in the disorder). Both cerebellar and basal ganglia blockade of the sodium pump were needed to cause dystonic symptoms, and lesioning of cerebellar output nuclei or the disynaptic cerebellar-basal ganglia link caused significant resolution of symptoms[20].

Clinical data

There are a substantial number of case reports linking dystonia to structural lesions of the cerebellum. However, there is considerable heterogeneity amongst cases with variable lesion location, aetiology and extent, type of dystonia produced, time interval between initial insult to onset of dystonia, and quality of clinical data. It has long been recognised in both adult and paediatric neurology that posterior fossa tumours can present with cervical dystonia [21, 22]. A review of 25 cases of secondary cervical dystonia with a range of aetiologies in adults revealed that structural lesions of the brainstem and cerebellum were the most frequent cause of cervical dystonia (44%), with basal ganglia lesions accounting for less (24%) of cases [23]. In two cases of cerebellopontine angle tumours, the cervical dystonia improved following successful removal of the tumour [24]. Focal limb dystonia has also been associated with cerebellar lesions. In an intriguing case, successful treatment of an isolated tuberculoma of the left cerebellar hemisphere led to parallel resolution of left arm dystonia [25]. Other cases document the emergence of late-onset oromandibular dystonia after bilateral cerebellar infarction, blepharospasm/torticollis after bilateral cerebellar infarction, and left hemidystonia following ipsilateral vertebral artery occlusion [26-28].

Patients with genetic degenerative cerebellar disorders (for example spinocerebellar ataxia type 3, SCA3) commonly demonstrate dystonia as part of their clinical phenotype, and sometimes dystonia may be the predominant presentation [29]. The neurodegeneration in such patients is widespread however, and the dystonia is usually assumed to be the result of basal ganglia rather than cerebellar degeneration. Pathological studies have indeed confirmed involvement of other motor system structures such as the pallidum and substantia nigra [30]. We have reported two separate series of patients with a syndrome of cervical dystonia and mild cerebellar ataxia (DYTCA) of undetermined etiology [31, 32]. Dystonia is the more prominent and disabling symptom in this disorder, with the cerebellar ataxia being relatively mild and slowly progressive. Imaging findings vary between patients

from cerebellar and brainstem atrophy to normality. Twelve further patients have been described in a separate series; although in these patients marked cerebellar atrophy (albeit with mild cerebellar signs) was the norm[33]. We have speculated whether the cerebellar pathology in our DYTCA patients contributes to (or perhaps is even wholly responsible for) the development of their dystonia. The electrophysiological studies that lend some support for this hypothesis are detailed below.

It is worth noting that in primary dystonia there is an absence of clear cerebellar signs on clinical examination, even when there is neurophysiological evidence to support cerebellar dysfunction[34]. This might be taken to support a more compensatory role for the cerebellum in some forms of primary dystonia, and at the very least tells us that the role of the cerebellum in dystonia is more complicated than simply a loss or gain of cerebellar function.

Functional and structural imaging data in dystonia

In vivo functional and structural imaging studies in dystonia can broadly be divided into studies of (i) grey and white matter structure and integrity (ii) neurotransmitters and (iii) brain metabolism at rest and during learning and motor tasks. The hereditary dystonias are particularly interesting as the incomplete penetrance of clinical manifestation in patients with mutations of DYT 1 or DYT 6 allow one to make distinctions between patterns of abnormality related to genotype and phenotype.

Voxel-based morphometry applied to high resolution MRI has demonstrated subtle changes in grey matter, with increases in putamal, internal globus pallidus and prefrontal cortex as a common pattern across different types of primary dystonia [35]. Both increases and decreases of cerebellar grey matter volume have been found with this technique in different types of dystonia and thus further studies are required to elucidate the significance of these observed changes [36-38]. Diffusion tensor imaging (DTI-MRI) can be used to assess microstructural white matter integrity with fractional anisotropy (FA) as a measure of axonal coherence [39]. Axonal integrity is reduced in the subgyral white matter of the sensorimotor area in both manifesting and non-manifesting DYT1 carriers, with additional FA reductions in the dorsal pons at its juncture with the superior cerebellar peduncle in manifesting subjects [40, 41] Additionally, DTI-MRI combined with probabilistic tractography techniques in DYT1 and DYT6 have demonstrated reduced connectivity of the cerebello-thalamic pathway near the dentate nucleus [42]. This was most pronounced in clinically affected mutation carriers compared with clinically unaffected mutation carriers. DTI-MRI in non-hereditary primary dystonias has also demonstrated white matter integrity abnormalities but as yet cerebellar connectivity has not been specifically studied [43-46].

As dystonia has traditionally been conceptualised as a basal ganglia disorder, abnormalities of dopaminergic neurotransmission have been investigated using radioligand binding. Decreased striatal D2 radioligand uptake has been demonstrated in various forms of primary dystonia including hand, cervical and cranial dystonia and patients with DYT1, DYT6 and DYT11 mutations regardless of clincial manifestation [47-50]. However, a recent study

suggests that D3 rather D2 receptor affinity is reduced in focal dystonia, thus further work with increasingly specific radioligands is needed to investigate whether this pattern is seen across other types of primary dystonia [51]. This work provides an interesting link with work presented above in animal models that cerebellar activity may directly modulate striatal dopamine (see above).

Alterations in regional brain function at rest can be measured using positron emission tomography (PET) with selective radioligands. Patients with sporadic and genetic forms of dystonia demonstrate relative increases in regional metabolic activity in the posterior putamen/globus pallidus, supplementary motor area (SMA) and cerebellum [52-54]. Elevated network activity persists during sleep in manifesting DYT1 carriers and is also present in non-manifesting DYT1 carriers [53]. Contrasting findings with regard to cerebellar metabolism have been found in DYT6 carriers, but all clinically affected DYT1 and DYT6 patients show relative metabolic increases in the pre-SMA and parietal association regions [55].

Sequence learning is a task that requires cerebellar processing [56]. Sequence learning ability and task-related brain activation is abnormal in non-manifesting carriers of the DTY1 deletion [57]. Sequence learning in conjuction with an equiperformance study design in this patient group resulted in overactivation of the lateral cerebellum, perhaps as a compensation for lack of recruitment of pre-frontal regions in order to achieve normal motor performance [58]. A normal motor-related activation pattern (NMRP) has been proposed by combining PET activation data with multivariate network modelling in control subjects [54]. The NMRP is characterised by contributions from the cortico-striato-pallidal-thalamocortical and cerebello-thalamo-cortical motor circuits. Groups of dystonic patients that have been studied to date (sporadic cervical dystonia and manifesting DYT1 and DYT6) demonstrate increased activity of the NMRP. Furthermore, the increased activity of the NMRP correlated with severity of dystonia and also microstructural changes observed by fractional anisotropy in cerebellar outflow as described above[54].

A large number of fMRI-BOLD studies have been conducted in patients with idiopathic primary dystonia and cerebellar abnormalities have repeatedly been described. Patients with musician's dystonia and focal hand dystonia show abnormal cerebellar activation during different tapping tasks [59, 60] and abnormal cerebellar activation is observed during writing in writer's cramp [61, 62]. Task-related activity in the cerebellar nuclei, posterior vermis, right paramedian cerebellar hemisphere and dorsal pons was however inversely related with the severity of hand dystonia and proposed to reflect secondary compensatory reorganization. Abnormal cerebellar activation in spasmodic dysphonia [63] during voice production and essential blepharospasm during eyeblinking [64] is also reported. Patients with cervical dystonia show BOLD signal increase in a number of brain regions including the cerebellum during passive movement [65].

Electrophysiological studies

Electrophysiological studies in dystonia have revealed a distorted balance between the excitatory and inhibitory circuitry of the sensori-motor system at various levels and there are abnormal responses to protocols inducing plasticity-like effects [66-69]. Investigation into the role of the cerebellum in these observed changes in dystonia is at an early stage. Firstly we compare the contrasting neurophysiological profiles seen in dystonia and cerebellar disorders. We then discuss eyeblink classical conditioning (EBCC), a paradigm that is heavily cerebellar dependent that is abnormal in dystonia. Finally we summarise recent work examining cerebellar inhibition in dystonia and areas for future investigation.

Studies investigating cortical excitability profile in primary dystonia using transcranial magnetic stimulation (TMS) have reported reduced cortical inhibition, observable as a relatively greater increase in motor evoked potentials (MEPs) with increasing stimulus intensities [70], shortening of the cortical silent period (CSP) [71] and reductions in short intracortical inhibition (SICI) [71-73]. A more limited number of studies have assessed the cortical excitability profile of patients with cerebellar lesions. The balance between cortical excitatory and inhibitory circuitry appears disturbed, but the shift is opposite to that seen in primary dystonia, with increases in motor cortical thresholds[74], abnormal prolongation of the CSP [75-77], reduced intracortical facilitation (ICF) [78, 79] and an increase in SICI [78]. We have compared measures of cortical excitability in five patients with DYTCA and found SICI to be increased [80], in contrast to typical primary dystonia, but similar to patients with cerebellar lesion [78]. Of interest, in myoclonus dystonia (DYT 11) the expected decrease in SICI seen in primary dystonia is absent [81]. Thus, cortical excitability profiles in dystonia are different with certain types (DYTCA, myoclonus dystonia) having patterns that more closely resemble patients with cerebellar disorders rather than typical primary dystonia. More work is clearly needed in this area, including testing response to plasticity protocols in these disorders to see if they are different from typical primary dystonia. Little is known about the role of the cerebellum in response to plasticity protocols, but preliminary work suggests that in cerebellar degeneration electrophysiological responses to plasticity inducing paradigms are normal [82].

Perhaps the most compelling electrophysilogical evidence for cerebellar involvement in dystonia is seen when studying EBCC. This is a paradigm of associative motor learning in which paired presentation of a conditioned (CS) and unconditioned stimulus (US) leads to the production of a conditioned eyeblink response (CR). Eyeblink classical conditioning has extensively been studied in humans and animals and is critically dependent on the cerebellum [83]. Patients with Parkinson's disease perform as well as healthy controls on EBCC [84], indicating that basal ganglia dysfunction does not necessarily impact significantly on this learning paradigm. In contrast, patients with adult onset focal dystonia have abnormal eyeblink classical conditioning [34]. Previous studies in animals and humans has revealed a cerebellar circuitry underlying EBCC in which the cerebellar cortical Purkinje cell (PC) receives convergent afferent information about the CS and US via two separate pathways

with an additional potential convergence upon the underlying interpositus nucleus (IN) [85]. It is noteworthy that structural imaging studies in dystonia identify grey matter abnormalities in the area of the cerebellar cortex that is involved in this circuit [86].

Purkinje cells in the cerebellar cortex have an inhibitory connection with the underlying dendate nucleus, which in turn displays a disynaptic excitatory connection through the ventral thalamus to the contralateral M1 [87]. Paired pulse transcranial magnetic stimulation (TMS) protocols can be used to study this pathway. In healthy subjects, a conditioning pulse delivered over the cerebellar cortex 5–7 ms prior to a test pulse over the contralateral M1 will result in reduction of the MEP amplitude relative to a test pulse given alone over this cortical area: "cerebellar brain inhibition" (CBI). This inhibitory effect is thought to arise from activation of Purkinje cells that will consequently inhibit the dentate nucleus and thus reduce the disynaptic excitatory drive from cerebellum to motor cortex [88, 89]. In eight patients with idiopathic focal limb dystonia, a cerebellar conditioning pulse had no effect on the test pulse MEP amplitude, SICI or ICF [90]. The authors hypothesized that the reduced cerebellar modulation of motor cortex excitability could arise through hyperactive purkinje cells in dystonia, that may be compensating for basal ganglia dysfunction. Another possibility is a reduced integrity of the cerebello-thalamo-cortical pathway in idiopathic primary dystonia, as has been described for hereditary primary dystonia [42].

It is possible that aberrant CBI in dystonia might interact with another phenomenon commonly reported in dystonia: abnormal surround inhibition. This is a muscle-specific modification of the excitability of the corticospinal pathway where just prior to and in the early phase of movement, muscles not involved in the planned movement but surrounding the active muscles show a decreased excitability due to active inhibition. Surround inhibition (SI) has repeatedly been reported to be disrupted in patients with primary dystonia and could account for the overflow of muscle activation seen in this movement disorder [91, 92]. The mechanism through which this inhibition is regulated has repeatedly been investigated but remains unknown and we have recently explored in healthy subjects whether there is a relationship between SI and CBI [93]. We did not observe a muscle specific modulation of CBI in parallel with SI, as CBI was reduced in both active and surround muscles at the onset of movement. However, the cerebellum has been proposed to be involved in the movement initiation processes and the observed change of the cerebellar inhibitory drive to the motor cortex at onset of movement is consistent with this. Although yet to be explored, it is possible that the abnormal CBI known to be present in dystonia could interfere with this process.

Regional cerebral blood flow in the ipsilateral cerebellum is negatively correlated with reaction time [94] and an increased reaction time is observed in patients with cerebellar dysfunction [95] as well as a decreased premovement corticospinal excitability [96]. It is intriguing that this increase in reaction time also applies to patients with primary dystonia [97] as well as the lack of MEP facilitation normally present before movement [98]. This altered release of motor programs in dystonia has been attributed to basal ganglia dysfunction, but a role for the cerebellum in these abnormalities is also conceivable.

Conclusions

Here we have outlined current evidence that explores a possible role for the cerebellum in dystonia. This field of exploration is still at an early stage and there are many unanswered questions. There is certainly *a priori* evidence from the important reciprocal anatomical connections between the cerebellum and basal ganglia to support the hypothesis that dysfunction in either structure might cause dysfunction or elicit a compensatory response in the other. Compensatory responses can also have their cost for the integrity of neural systems, seen for example in increases in activity of motor areas in the non-stroke hemisphere after stroke which may have an unwanted inhibitory effect on the stroke hemisphere and impair recovery [99]. This increases the complexity of interpreting abnormalities in the cerebellum revealed by experimental studies in dystonia patients.

In certain types of dystonia cerebellar dysfunction may play a primary role in the pathology of the disorder. Here the data from clinical cases with cerebellar lesions and dystonia, and from patients with DYTCA or myoclonus dystonia who have the electrophysiological "profile" of patients with cerebellar degeneration rather than typical primary dystonia are noteworthy. However, the lack of traditional "cerebellar signs" in most patients with dystonia points more strongly to a compensatory role for the cerebellum in most forms of primary dystonia. This is in line with functional imaging data showing increased cerebellar dependence for sequence learning in dystonic patients [58]. Even the finding of abnormal eyeblink conditioning in primary dystonia might be explained by a disruption of cerebellar function induced by compensatory changes in this structure that are induced by primary basal ganglia dysfunction.

Work in this exciting area has only just begun, but already it is clear that the historical reputation of dystonia as a mysterious and constantly changing concept is likely to continue. In the future, the cerebellum is likely to have a key place within pathophysiological models of this enigmatic disorder.

Chapter 2

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3.1

A distinctive pattern of cortical excitability in patients with the syndrome of dystonia and cerebellar ataxia.

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Abstract

The syndrome of dystonia and cerebellar ataxia (DYTCA) is a recently described condition where cervical dystonia and mild cerebellar ataxia are the major clinical features. Here we attempted to explore the pathophysiology of this condition by comparing measurements of cortical excitability between patients with DYTCA, typical primary dystonia and healthy controls.

Motor threshold, active MEP recruitment and CSP duration were measured and the excitability of the intracortical inhibitory and excitatory circuits was assessed at rest using a paired pulse protocol.

We identified a distinctive pattern of cortical excitability in DYTCA patients different from that found in primary dystonia, namely hyperexcitable short-interval intracortical inhibition.

DYTCA patients have a noticeably dissimilar excitability profile from patients with primary dystonia.

A tendency for increased SICI has been previously described in cerebellar syndromes and the altered excitability profile seen in these patients is therefore possibly a consequence of the cerebellar dysfunction in DYTCA. A direct link between reduced intracortical inhibition and dystonia has recently been questioned and our results additionally suggest that reduced motor cortex inhibition is not a prerequisite for dystonia to occur.

Introduction

The basal ganglia are historically implicated as the key player in the pathophysiology of dystonia but there is increasing interest in a potential additional role of the cerebellum [1-3]. The dentate nucleus of the cerebellum provides a disynaptic input to the striatum [4] and communication may also arise from convergence of separate thalamo-cortical connections that influence cortical excitability.

We previously published two separate series of a total of 11 cases with a new syndrome of mostly cervical dystonia and cerebellar ataxia of undetermined etiology [5, 6]. The cerebellar ataxia in this syndrome is relatively mild and slowly progressive, with the dystonia being more prominent and disabling. Imaging findings vary between patients from cerebellar and brainstem atrophy to normal. Le Ber et al. previously identified 12 patients with an unusual phenotype that consisted of mainly laryngeal dystonia and a mild cerebellar syndrome, with marked cerebellar atrophy on brain MRI in most patients [7], which may be a similar syndrome to that described by ourselves.

Secondary dystonia can arise from structural cerebellar lesions due to stroke [8] and tuberculoma [9]. Consequently, one wonders whether the cerebellar pathology in DYTCA patients contributes to (or is even wholly responsible for) the development of their dystonia. As an initial investigation into this question we compared measures of cortical excitability in patients with DYTCA with patients with typical primary focal/segmental dystonia and healthy subjects.

Methods

Five DYTCA patients, 11 patients with primary focal or segmental dystonia (DYT), and 10 healthy subjects were recruited after informed consent (Table 1). The study was approved by the joint ION/NHNN research ethics committee and participants gave their informed consent to take part. For further clinical details and results of auxiliary investigations, we refer to our previous papers [5, 6]. In short, these patients have had a full work-up for diseases that can lead to the combined presence of ataxia and dystonia, but no cause was found. The etiology of this syndrome is therefore unknown, but a strong genetic component is suspected.

Transcranial magnetic stimulation (TMS) was delivered using a standard 70 mm figure-of-eight coil, connected to one (single pulse) or two (paired pulse) Magstim 200 monophasic stimulators, over the M1 area at the optimal position for eliciting motor evoked potentials (MEP) from the first dorsal interosseous muscle (FDI). Ag/AgCl electrodes were used to record EMG activity. Signals were filtered (30 Hz–1 kHz) and then stored on computer via a Power 1401 data acquisition interface (Cambridge Electronic Design Ltd., Cambridge, UK). Analysis was carried out using Signal Software (Cambridge Electronic Design). The hemisphere contralateral to the most affected body part was stimulated in patients. In healthy subjects, the dominant M1 was tested.

Table 1 | Subject characteristics of DYTCA-patients, DYT-patients and healthy controls are shown in this table.

	Number	Age	Female	Mean disease duration	Type of dystonia	
					Pure torticollis	Torticollis with mild hand/arm involvement
DYTCA-patients	5	43.4 ± 15.3	2	8.2 (range 3–18)	0	5
DYT-patients	11	51.3 ± 10.0[a]	9	14.5 (range 3–32)	6[b]	5
Healthy controls	10	43.5 ± 15.1	5			

a   Patients with primary dystonia were slightly older but overall age and sex were not significantly different among groups.
b   One patient with laryngeal dystonia.

TMS measures included active (AMT) and resting motor threshold (RMT) according to standard definitions [10]; recruitment of MEP amplitude during slight background activation starting with 100% RMT and increasing stepwise by 10% RMT up to 150% RMT; the cortical silent period (CSP) at the same stimulation intensities. For all active studies subjects were encouraged to maintain a steady background contraction of about 10% of the maximum by visual and auditory feedback.

The excitability of the intracortical inhibitory and excitatory circuits was assessed at rest using a paired pulse protocol with the conditioning pulse at 80% AMT and the test pulse adjusted to elicit an MEP of 0.5–1 mV. Short interval intracortical inhibition (SICI) was measured using interstimulus intervals of 2 and 3 ms; for intracortical facilitation (ICF) interstimulus intervals (ISI) of 7, 10 and 15 ms were tested and averaged after initial testing of these individual ISIs did not reveal significant differences (see Results section); previous work suggests that the mechanism of facilitation at this range of ISIs is similar [11] and different individuals may show maximum ICF at a different interval [12]. To test the excitability of the inhibitory circuits we constructed a recruitment curve for SICI using 4 different conditioning intensities: 70, 80, 90 and 100% AMT at the 2 ms interval; in healthy subjects inhibition appears at 80% and is usually maximal between 90% and 100% AMT [13].

Statistical analysis

TMS measures were logarithmically transformed where necessary for use of parametric tests. Two-factor ANOVAs were used to compare TMS measures among groups. The between subject factor was always GROUP (levels: DYTCA, DYT and CONTROL); the within subject factor was either stimulus intensity (SI) or interstimulus interval (ISI). One-factor ANOVAs were subsequently used to investigate significant main effects. Bonferronni's correction was

used for post hoc within subject comparisons; for between subject comparisons we used Gabriel's test for significantly unequal samples. Significance level was set at 0.05.

Approximately 50% of the patients with primary dystonia had arm/hand involvement (DYT*t-plus*), as did all the DYTCA patients (Table 1); in order to clarify the importance of arm involvement in our findings we performed one-way ANOVAs with between-subjects factor SUBGROUP (DYT*t-plus*, DYTCA and controls); only SICI and the SICI recruitment curve were tested for this.

Results
Corticospinal excitability
No significant group differences were observed for motor threshold, active MEP recruitment or increase of CSP duration with increasing stimulation intensity.

Paired-pulse intracortical excitability
A two-factor ANOVA with factors GROUP and ISI (levels 2, 3 and 7–15 ms) was used. Initial testing did not reveal any significant differences at long ISIs, i.e. 7, 10 and 15 ms, either between or among groups (values are shown in Table 2). The ANOVA revealed a significant main effect of ISI [$F_{(2, 46)}$ = 92.4, $p < 0.001$], no main effect of GROUP and a significant ISIxGROUP interaction [$F_{(4, 46)}$ = 2.9, $p < 0.03$]. Subsequent one-way ANOVAs revealed a group difference at the 3 ms interval [$F_{(2)}$ = 3.6, $p = 0.04$]. DYTCA patients tended to show stronger inhibition compared to DYT patients ($p = 0.056$) but not when compared to normal controls. No trends were noticed between DYT patients and controls (Figure 1A).

SICI recruitment curve at 2 ms
Two-factor ANOVA with factors GROUP and SI (levels 70%, 80%, 90% and 100% AMT) showed a significant main effect of SI [$F_{(3, 66)}$ = 17.9, $p < 0.001$], no main effect of GROUP, but a significant SIxGROUP interaction [$F_{(6, 66)}$ = 4.8, $p < 0.001$]. Subsequent one-way ANOVAs using Gabriel's post hoc test revealed significantly stronger SICI for DYTCA patients at 70% AMT compared to the DYT ($p < 0.001$) and the control group ($p = 0.01$); there were no differences between DYT and healthy controls (Figure 1B).

Subgroup analyses
Paired-pulse intracortical excitability
A significant main effect of SUBGROUP (levels: DYTCA, DYT*t-plus*, control) [$F_{(2, 19)}$ = 5.4, $p = 0.015$] was found when comparing intracortical excitability at the ISI of 3 ms. Post hoc comparisons revealed that DYTCA patients showed significantly stronger SICI compared to DYT*t-plus* patients ($p = 0.015$) but not compared to controls; moreover, DYT*t-plus* patients showed weaker SICI compared to controls ($p = 0.049$). No main effects were seen for the 2 ms ISI.

Table 2 | TMS measurements of DYTCA-patients, DYT-patients and healthy controls are shown in this table. RMT (resting motor threshold) and AMT (active motor threshold) stimulation intensity. Size of the MEP in mV elicited at different stimulation intensities. CSP expressed as ms elicited at different stimulation intensities. ICF displayed as the amount of excitation as percentage change in MEP-amplitude for different ISI. ISI = interstimulus interval.

	DYTCA-patients	DYT-patients	Healthy controls
RMT	40.8 ± 11.2	39.1 ± 5.5	38.4 ± 7.4
AMT	35.4 ± 11.1	31.5 ± 5.3	31.0 ± 5.8
MEP 100% RMT	1.2 ± 0.9	1.8 ± 1.1	2.3 ± 1.0
MEP 110% RMT	2.1 ± 1.2	3.3 ± 1.4	4.0 ± 2.2
MEP 120% RMT	2.9 ± 1.3	4.7 ± 1.9	5.4 ± 2.6
MEP 130% RMT	4.1 ± 2.1	5.2 ± 2.7	6.6 ± 3.0
MEP 140% RMT	4.5 ± 2.2	5.7 ± 2.7	6.6 ± 2.7
CSP 100% RMT	98.0 ± 61.1	68.6 ± 23.8	81.8 ± 17.9
CSP 110% RMT	135.2 ± 51.7	91.0 ± 28.5	108.5 ± 20.2
CSP 120% RMT	159.1 ± 45.4	117.8 ± 36.3	130.0 ± 17.1
CSP 130% RMT	170.8 ± 38.3	141.2 ± 31.1	149.9 ± 20.8
CSP 140% RMT	182.5 ± 40.3	159.3 ± 30.1	168.7 ± 18.3
ICF 7 ISI	1.2 ± 0.5	1.1 ± 0.3	1.3 ± 0.3
ICF 10 ISI	1.1 ± 0.3	1.2 ± 0.3	1.4 ± 0.4
ICF 15 ISI	1.1 ± 0.4	1.1 ± 0.2	1.3 ± 0.1
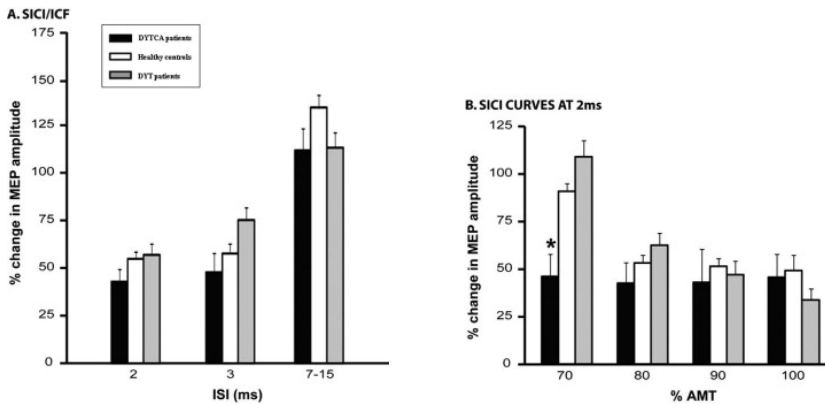
Figure 1 | (A) SICI and ICF recruitment curve for patient groups and controls. Y-axis displays the amount of inhibition (interstimulus interval 2 or 3 ms) or excitation (7–15 ms) as percentage change in MEP-amplitude. X-axis displays interstimulus interval in ms (2, 3, 7–15). ISI = interstimulus interval. (B) SICI recruitment curve at 2 ms for patient groups and controls. Y-axis displays the amount of inhibition as percentage change in MEP amplitude. X-axis shows stimulus intensity levels (70%, 80%, 90% and 100% of AMT). *P < 0.05. The error bars represent standard errors.

SICI recruitment curve at 2 ms

One-way ANOVAs were separately performed at SI levels of 70%, 80%, 90% and 100% AMT. A significant main effect of SUBGROUP ($F(2, 18) = 12.7$, $p < 0.001$) was found for SI 70% AMT; post hoc tests confirmed that DYTCA patients showed significantly stronger SICI compared to controls ($p = 0.012$) and DYT*t-plus* patients ($p < 0.001$; the difference between DYT*t-plus* patients and controls was in this case just significant ($p = 0.05$). These results suggest that reduced SICI is more likely in patients with primary torticollis when there is a degree of arm/hand involvement.

Discussion

The main finding of this study is that DYTCA patients have a significantly lower threshold for evoking SICI in comparison with dystonia patients (DYT) and with healthy controls.

The reduced SICI threshold in DYTCA was in contrast to the normal threshold for recruitment of MEPs. The fact that the maximum recruited amount of SICI was similar to that in healthy subjects suggests that there has been a leftwards shift in the recruitment curve of SICI. There are two possible explanations for this, the most obvious being a higher excitability of the GABAergic system responsible for SICI in DYTCA patients. However, it could also be that the composition of the test response is subtly different in the patient group. The TMS test pulse evokes a series of I-wave volleys in the corticospinal tract that summate at spinal motor neurones to produce the MEP. SICI preferentially suppresses the I3 and later volleys; if there are more late volleys in patients, perhaps balanced by reduced numbers of I1 and I2 volleys, then they will appear to have more sensitive SICI. A similar explanation has been put forward for the usual reduction in SICI that is reported in primary dystonia [14]. The precise make up of I-wave volleys in different patient groups has never been estimated so currently we cannot distinguish between changed excitability of the GABA system and changed recruitment patterns of the MEP. It is important that these issues are tested in future studies considering the implications they have for interpretation of the pathophysiology of these conditions. Nevertheless, the data here do indicate the need to examine SICI at a range of different conditioning intensities since examination at the usual 80% AMT failed to reveal any significant difference between participants. It would have been interesting to see whether similar or even more robust differences could be demonstrated at the 3 ms ISI, as some trends for increased SICI were seen at the 3 ms interval using a conditioning intensity of 80% AMT.

Interestingly, a tendency for increased SICI has been previously described in cerebellar syndromes. Liepert et al. [15] found increased SICI in patients with a recent infarct in the SCA territory. Very few studies have looked at patients with degenerative cerebellar syndromes; in most of them SICI has been normal, but note that the threshold for SICI was not studied [16, 17]. There has however been a report of decreased SICI in patients with familial cortical myoclonic tremor, in which the pathological correlate has proven to be cerebellar cortical degeneration [18]. Even more, Tamburin et al. [19] found an increase in long-interval

intracortical inhibition, a TMS measure also thought to reflect activity in inhibitory pathways, although the underlying mechanisms are thought to be different; it is interesting, however, that the differences were again more evident using interstimulus intervals that normally result in less than maximal inhibition. We therefore infer that the cortical excitability profile we found in our DYTCA patients could reflect the cerebellar involvement and with that the cerebellar modulation of the functional connections in the motor cortex. It would be useful to study more patients but this is made difficult by the rarity of the condition. As there are no neuropathology reports on DYTCA, we cannot exclude the possibility that the involvement of other brain regions than the cerebellum contribute to the findings presented here.

In addition, the results also raise the question of the relationship between reduced SICI and primary dystonia. Reduced SICI in dystonia was previously linked directly to a general lack of inhibition in movement commands, leading to co-contraction and overflow of activity to muscles uninvolved in the task. However, reduced SICI has also been observed in the unaffected limbs of patients with focal dystonia [20], in fixed dystonia [21], and even in unaffected carriers of dystonia gene mutations [22]. In this study, patients with primary dystonia did not show a clear reduction in SICI but did show less SICI at the left end of the recruitment curves, i.e. at low SICI thresholds, as reported previously [23]; the subgroup analyses suggests that this could be related to the clinical heterogeneity of our dystonia group (cervical dystonia with and without arm involvement), in other words arm/hand dystonia is more likely to be associated with reduced SICI measures from the hand muscles. This is in agreement with a previous report from Hanajima et al. showing reduced intracortical inhibition in the sternocleidomastoid muscles but normal inhibition in the first dorsal interosseous muscles [14]. We can add the present observations to this ongoing debate: that is, patients with DYTCA and overt dystonia of the arm have, if anything, increased SICI rather than reduced SICI, suggesting that reduced SICI is not a sine qua non in dystonia. It is however possible, that the cerebellar involvement has concealed the dystonic SICI profile. Until we know the reason for the changes in SICI in these conditions (i.e. whether it is due changes in the GABA system or to changes in I-waves), it is difficult to speculate on their functional relevance. For example, it may well be that it is not the resting level of SICI that is important, but the control of inhibition during movement.

In conclusion, we have identified subtly enhanced SICI in DYTCA patients that may reflect cerebellar dysfunction. This suggests that dystonia in DYTCA might be due to mechanisms that are different from those in primary dystonia, perhaps with a strong cerebellar contribution, but further work is needed.

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3.2

Cerebellar brain inhibition is decreased in active and surround muscles at the onset of voluntary movement.

Abstract

Highly selective activation of the desired muscles for each movement and inhibition of adjacent muscles is attributed to surround inhibition (SI) which differentially modulates corticospinal excitability in active and surrounding muscles. Cerebellar brain inhibition (CBI) is another inhibitory neuronal network which is known to be active at rest and during tonic muscle contraction. The way in which CBI may be modulated at movement onset and its relationship with SI has not previously been investigated. We assessed motor evoked potential (MEP) size and CBI in first dorsal interosseus (FDI) and abductor digiti minimi (ADM) muscles at rest and during a simple motor task where FDI was an active muscle and ADM was not involved in the movement (surround muscle). At onset of movement, MEP size in ADM was significantly suppressed, confirming the existence of SI. In contrast, CBI in both muscles was found to be significantly decreased at the onset of the movement. This was confirmed even after adjustments for changes in MEP size occurring due to onset of muscle activity in FDI and the effects of SI in ADM. Our findings fail to functionally link SI with CBI, but they do indicate a non-topographically specific modulation of CBI in association with initiation of voluntary movement.

Introduction

Preparation and execution of voluntary movement is a complex process involving activation of numerous neuronal components and networks. One important component of this process is surround inhibition (SI) [1], where muscle-specific modification of the excitability of the corticospinal pathway occurs. Just prior to and in the early phase of movement, muscles not involved in the planned movement that physically surround the active muscle show a decreased excitability, thought to be due to active inhibition. This inhibition reaches its maximum peak at the onset of the movement [1, 2]. The likely clinical importance of SI is supported by several electrophysiological studies in dystonic patients which reveal that the involuntary co-contraction of hand muscles that occurs in this condition is associated with a disruption of SI [3, 4]. Several different factors including force level and task difficulty modulate the amount of SI [2, 3], but the mechanism through which this inhibition is regulated is unknown. Intracortical inhibitory processes including short intracortical inhibition (SICI), long intracortical inhibition (LICI) and intracortical facilitation (ICF) do not appear to have any direct regulatory role on SI [1].

Cerebellar brain inhibition (CBI) is an inhibitory circuit which is thought to be mediated through the dentato-thalamo-cortical pathway [5, 6]. Using transcranial magnetic stimulation (TMS), the size of the motor evoked potential elicited by a TMS pulse over one hand motor area is significantly reduced by a TMS pulse, delivered over the contralateral cerebellar hemisphere, 5-7 ms earlier. CBI occurs at rest but has been found to be reduced in hand muscles during tonic activation of proximal arm muscles [7]. It is not known how CBI may be modulated in active and surround muscles during movement preparation and at movement onset when SI is most prominent. Here, we aimed to probe the relationship between SI and CBI. We hypothesised that, if such a relationship existed, CBI during movement initiation would be differentially modified in an active and surround muscle, being reduced in the contracted muscle and increased in the surrounding muscles.

Methods

Participants

16 healthy volunteers (mean age 29 ± 9 years; range 22–52 years; 9 men and 7 women) participated in the study after giving their written informed consent. All of them, except for one, were right-handed and none of them had any history of neurological disease. The study was approved by local ethics committee and conducted in accordance with regulations laid down in the Declaration of Helsinki.

Electromyographic recordings

Electromyographic (EMG) activity was recorded from right first dorsal interosseus (FDI) and abductor digiti minimi (ADM) muscles using a pair of Ag–AgCl surface electrodes in a belly-tendon montage. Ground electrode was placed above the styloid process of the right ulna. The EMG signal was amplified (1000×) and band-pass filtered (bandwidth 20–2,000 Hz) with

a Digitimer D360 amplifier (Digitimer Ltd, UK), digitized at a sampling rate of 5 kHz (CED 1401 laboratory interface; Cambridge Electronic Design, Cambridge, UK) and fed into a laboratory computer for storage and off-line analysis. Data were analysed using SIGNAL software V4.00 (Cambridge Electronic Design, Cambridge, UK).

Motor task

During the experiments, the subjects were sitting in a comfortable chair with their right hand resting on a desk. While their hand was lying flat and relaxed on the desk, the tip of their index finger was placed on a small button. They were asked to briefly press the button after a 'go' signal (an auditory tone) with a self-paced delay, by flexing their index finger in the metacarpo-phalangeal joint. FDI is a synergist rather than a primary muscle for this movement but previous studies have shown that this movement induces activation of FDI and suppression of ADM through SI [1]. At the beginning of the experiment, we measured the individual maximum EMG activity which could be produced in FDI by briefly pressing the button. Then we asked the subjects to perform the same brief movement with 10% of their maximum EMG activity. They were also asked to keep their ADM muscle totally relaxed while they were doing the task. Visual feedback of the EMG activity from both muscles (FDI and ADM) was displayed on a screen in front of the subjects. Training sessions before the start of the experiments were needed for a consistent performance of the desired movement to be attained by the subjects with EMG activity in ADM not to exceed 100 µV. We examined SI and CBI at rest and at the onset of the movement.

Transcranial magnetic stimulation

A figure-of-eight shaped coil (external loop diameter of 9 cm) connected to a monophasic Magstim 200 stimulator (Magstim Co, Carmarthenshire, Wales and UK) delivered TMS over the left motor cortex. The intersection of the coil was positioned tangentially on the scalp over the left motor cortex at the optimal site for eliciting motor evoked potentials (MEP) of maximal amplitude in the right ADM. The handle of the coil was pointing backwards and laterally at a 45° angle to the saggital plane in order to induce trans-synaptically a posterior–anterior directed current in the brain to activate the corticospinal tract [8, 9]. The hot spot was marked with a felt pen in order to ensure consistent coil position during the experiment. For the assessment of SI, single TMS pulses were delivered at rest and at the onset of the movement. TMS at movement onset was achieved using the peri-triggering function of SIGNAL software which was set to trigger TMS immediately when EMG activity in right FDI above 100 µV was detected. The intensity of the stimulation was set to evoke MEPs with average peak-to-peak amplitude of approximately 0.5 mV–1 mV at rest in ADM, which was found from previous studies to be ideal for CBI assessment [6, 7, 10, 11].

The cerebellar conditioning stimulus (CS) was delivered over the right cerebellar hemisphere with a double-cone coil (110 mm mean diameter). This type of coil has been found in previous studies to be the most efficient for cerebellar stimulation in CBI paradigms [6, 12]. The exact

position of the coil was 3 cm lateral to the inion on the line connecting the inion and the external auditory meatus [5, 6, 11]. The current of the coil was directed downwards in order to induce an upwards current in the cerebellar cortex [6, 10, 11]. In line with previous studies on CBI, cerebellar stimulation intensity was set at 5% below the pyramidal tract active motor threshold (AMT) [11, 13], in order to minimise confounding effects due to brainstem or nerve root stimulation [6, 14]. The AMT for pyramidal tract was measured with the coil positioned on the inion while subjects maintained background EMG activity of 10% of their maximum force in FDI [11]. Five trials of each intensity were averaged and the minimum intensity which induced MEP responses of 50 μV or more above the background activity was considered to be the pyramidal tract AMT. Threshold was determined to the nearest 5% of the stimulator output [7, 11]. The Interstimulus interval (ISI) between the CS and the test stimulus (TS) of motor cortex was set at 5 ms. This ISI was found by Saito et al. to be the optimal for CBI and its effect is attributed to cerebellar cortex stimulation rather than stimulation of other peripheral structures (e.g. muscle, nerve, plexus) [5, 7, 11, 12]. For the assessment of CBI at the onset of the movement, we used the peri-triggering function of SIGNAL software set to elicit the CS immediately after the detection of EMG activity above 100 μV in FDI followed 5 ms later by the TS.

Experimental design

There were four blocks of experimentation: assessment of MEP size at rest (single pulses), assessment of MEP size at movement onset (single pulses), CBI at rest (paired pulses), CBI at movement onset (paired pulses). For each of the blocks, 15 stimulation trials were recorded. In the blocks assessing MEP size or CBI at movement onset, we also included 15 trials with no stimulation mixed with the 15 stimulation trials in a randomised fashion. This ensured that subjects continued to perform the movement during these blocks and were not aware of when a stimulation trial might occur. The order of the blocks was also randomised between participants.

Statistical analysis

Peak-to-peak MEP amplitude for each trial was measured off-line and the average amplitude in 15 trials was calculated for each session. CBI was expressed as the ratio of conditioned MEPs to unconditioned MEPs. SI was expressed as the ratio of MEP amplitudes during peri-triggered trials to MEP amplitudes in control trials. The effects of SI and CBI were evaluated through repeated measures analysis of variance (ANOVA). Wherever significant interactions were observed, we did post hoc tests with Bonferroni corrections to further analyse the results. Statistical significance was set to $P < 0.05$. Unless otherwise stated all results are expressed as mean values ±1 standard deviation (SD).

Results

None of the subjects reported side effects from the experiments. A total of 16 participants completed the study. Seven further participants (5 men and 2 women), recruited for the study, were unable to complete the experiments because either they found cerebellar stimulation too uncomfortable or after a practice session of 30 min they could not constantly maintain their right ADM quiet enough (background EMG activity less than 100 µV) while they were performing the task.

Surround inhibition

Two-way repeated measures ANOVA revealed significant difference of MEP amplitudes in ADM and FDI at rest and on the onset of the movement. We found significant main effects of MUSCLE (levels: ADM and FDI) ($F_{(1,15)} = 78.20$, $P < 0.01$), and CONDITION (levels: Rest and Onset of the movement) ($F_{(1,15)} = 88.66$, $P < 0.01$) and their interaction MUSCLE × CONDITION ($F_{(1,15)} = 134.55$, $P < 0.01$). Post hoc pairwise comparisons demonstrated significant mean difference for the factor MUSCLE = 3.80 (95% CI = 2.88–4.72) and for the factor CONDITION = 2.11 (95% CI = 1.64–2.59) (Figure 1, 2). The significant suppression of ADM MEP size confirms the existence of surround inhibition in our participants.
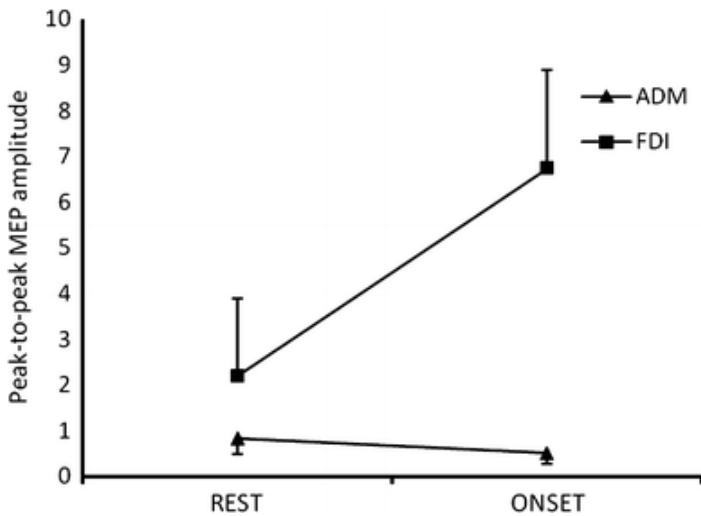
Figure 1 | Surround inhibition. FDI is highly facilitated ($P < 0.01$) at the onset of the movement. Non-active ADM is suppressed due to SI ($P < 0.01$). Error bars indicate SD.
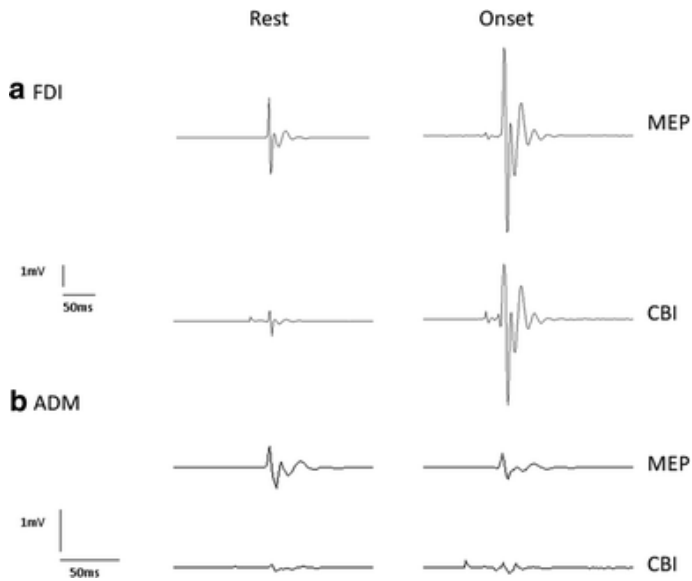
Figure 2 | Example trace of raw data from one subject showing a increase in FDI MEP and b decrease in ADM MEP at the onset of movement with a corresponding decrease in CBI in both muscles. Note that the scales for traces recorded from FDI and ADM are different for the sake of clarity of the figure.

Cerebellar brain inhibition

We expressed CBI as the ratio of MEP amplitudes of conditioned responses to MEP amplitudes of unconditioned responses. An increase in this ratio therefore indicates a reduction of CBI. Repeated measures ANOVA revealed significant effects of the factor CONDITION (levels: Rest and Onset of the movement) ($F(1,15) = 6.48$, $P = 0.02$) and no significant effect of the factor MUSCLE ($F(1,15) = 0.22$, $P = 0.65$) or their interaction MUSCLE × CONDITION ($F(1,15) = 0.08$, $P = 0.78$) (Figure 2, 3). Post hoc pairwise comparisons showed significant mean difference of the factor CONDITION = 0.27(95%CI = 0.04–0.50) due to a reduction in CBI at the onset of the movement compared to CBI at rest in both muscles.

MEP matching

MEP sizes in FDI and ADM changed significantly at movement onset, due to muscle activation (in FDI) and SI (in ADM). In order to determine, if the change in MEP size itself might be responsible for any changes in level of CBI (Ugawa et al. 1995) at the onset of movement, we performed further recordings of CBI at the onset of the movement in 6 subjects with adjusted TS intensity. Firstly, we increased the intensity of the motor cortex stimulation to a level at which the MEP responses in ADM elicited by the TS alone at the onset of the movement were of the same amplitude as the MEP responses we recorded at rest. Then, we used this new intensity to record CBI at the onset of the movement. We did the same for FDI but this time we decreased the TS intensity in order to achieve MEPs at the onset of the movement of the
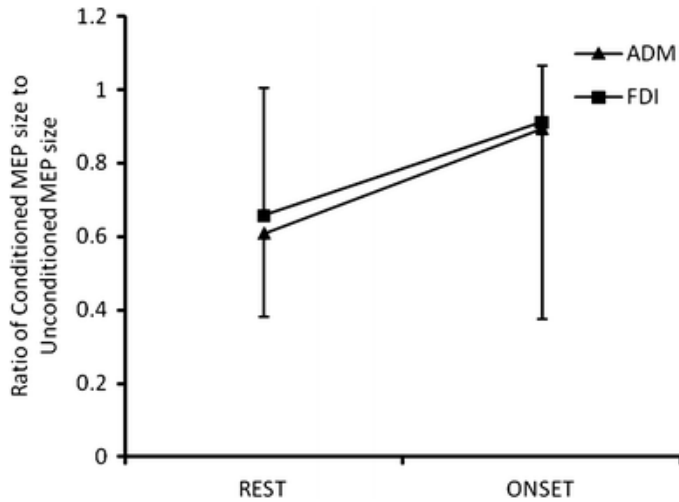
Figure 3 | Significant decrease of CBI was found in both muscles (P = 0.02). CBI reduction is not significantly different in the two muscles (P = 0.65). Error bars indicate SD.

same amplitude as the ones we recorded when the muscle was relaxed (Mean TS intensity for the main experiment was 52% of the maximum output of the stimulator—range from 36 to 70%, Mean TS intensity for ADM matching experiment was 55% of the maximum output of the stimulator—range from 39 to 75%, Mean TS intensity for FDI matching experiment was 34% of the maximum output of the stimulator—range from 23 to 45%). Paired samples t-test showed that there was no significant difference between the MEP size at rest and the matched MEP size at the onset of the movement for both ADM (t(5) = 1.27, P = 0.27) and FDI (t(5) = 0.34, P = 0.75). Repeated measures ANOVA revealed no significant effect of the factors GROUP (levels: CBI at movement onset, CBI at movement onset with matched MEPs) (F(1,5) = 3.14, P = 0.14) or MUSCLE (levels: ADM, FDI) (F(1,5) = 0.11, P = 0.75) or their interaction GROUP × MUSCLE (F(1,5) = 3.10, P = 0.14) (Figure 4). This indicates that the reduction in CBI observed in ADM and FDI at the onset of movement cannot simply be explained by the change in MEP size occurring at this time in ADM and FDI.
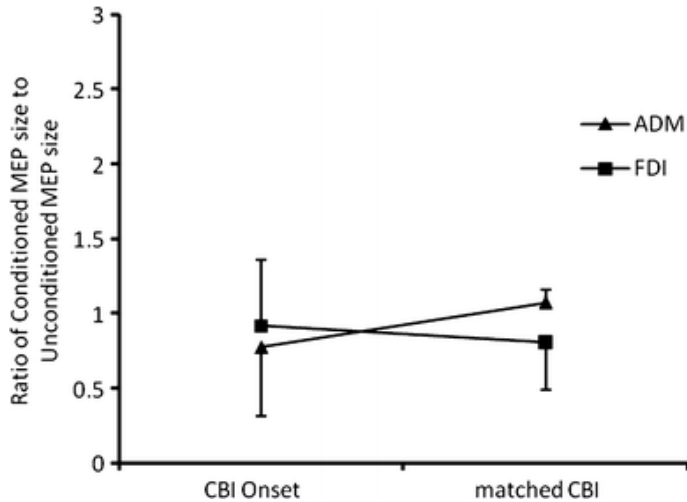
Figure 4 | MEP matching on the onset of the movement. There is no significant difference between CBI at movement onset and CBI with TS size adjustment. Increased TS intensity was used for matched CBI in ADM and decreased TS intensity for matched CBI in FDI. Error bars indicate SD.

Discussion

We have demonstrated that CBI is reduced in both active and surround muscles at the onset of movement. While our initial hypothesis that there may be muscle-specific modulation of CBI at onset of movement in parallel with SI was not confirmed, the data do provide novel evidence of a change in cerebellar inhibitory drive to the motor cortex at onset of movement. Our data extend the findings of one previous study that has explored the effect of muscle activity on CBI. Pinto and Chen (2001) compared CBI in FDI at rest and when FDI was relaxed, but subjects also maintained their ipsilateral or contralateral arm outstretched. Activation of ipsilateral proximal arm muscles led to a significant reduction of CBI in FDI. However, this study only examined the effect of tonic muscle contraction in a distant muscle, and any possible effects of prolonged shoulder extension on the MEP size in the otherwise relaxed FDI were not controlled for [7].

In both active FDI and the surround muscle ADM, we identified the same amount of reduction of CBI at movement onset, the time at which the effects of SI are most prominent [1, 2]. Identical CBI reduction in both active and surrounding muscles makes it unlikely that this specific cerebellar inhibitory mechanism is responsible for driving inhibition of surround muscles. What might, therefore, be the contribution of this reduction in cerebellar inhibitory drive to movement preparation and execution?

There is evidence to show that cerebellum is involved in movement initiation processes. Changes in the blood flow in the ipsilateral cerebellar hemisphere are associated with changes in reaction time of voluntary movement [15]. In addition, patients with cerebellar dysfunction have increased reaction time [16] and moreover ischaemic lesions in the

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cerebellum lead in decreased premovement corticospinal excitability [17]. These findings imply that the cerebellum may have a role in movement initiation, and therefore it is possible that modification of CBI could contribute to the implementation of this function. Furthermore, according to the model proposed by Houk and Wise (1995) for planning and controlling movement, the triggering process for a movement may be different from the programing process. In this regard, the cortical-cerebellar loop is hypothesised to be involved in triggering the initiation of the action command [18]. Within this model, our finding of a non-muscle-specific CBI reduction at the onset of the movement fits with a triggering role for the cerebellum through withdrawal of motor cortex inhibition. In contrast, SI may be more important for the programing process through muscle-specific regulation of corticospinal excitability. It would be of interest to further explore the time course of modulation of CBI in the preparation and execution phases of movement.

Although the role of afferent cerebellar input in voluntary movement initiation and execution is not well understood, it is known that CBI still exists even when cerebellar input pathways are damaged [12]. Lack of CBI dependence on input from the periphery implies that it is highly unlikely for CBI to have a corrective role, but it does not exclude the possibility that it has a role in preparedness for possible future corrections. Reduction of inhibition in both active and surrounding muscles at the onset of the movement might be responsible for bringing the motor system into a state where future corrections can be efficiently performed even if they implicate surrounding muscles, for example to allow for rapid adjustments to improve movement stability.

During the MEP recordings, TMS stimulation was given immediately on the onset of the movement (0 ms delay), when EMG activity exceeded the peri-triggering threshold. For CBI recordings at the onset of the movement, the CS was given at the onset of the movement (0 ms delay), and the TS 5 ms later (5 ms delay). Although this introduces a small time difference in the two recordings, previous studies examining SI have found that the inhibitory effect on the surround muscle only begins to disappear 100 ms after the onset of the movement [1]. Therefore, a delay of 5 ms in the timing of the TS delivery is highly unlikely to have had any significant effect on the results. We included one left handed subject and are aware that surround inhibition has been reported to be asymmetric [19], being less marked on the non-dominant side. However, the results of this subject with regard to SI (MEP amplitude in ADM at rest/MEP amplitude in ADM on the onset = 0.59) and change in CBI at movement onset (MEP amplitude elicited by conditioned stimulation/MEP amplitude elicited by unconditioned stimulation in ADM at rest = 0.88, on the onset = 1.16, in FDI at rest = 0.87, on the onset = 0.97) were of a similar direction and magnitude to the group means.

In conclusion, we report for the first time how CBI is modulated at the onset of a brief movement in the active FDI muscle and the surrounding ADM muscle. This does not provide evidence of a functional link between CBI and SI. Instead, we found significant non-topographically specific reduction in the excitability of cerebello-thalamo-cortical inhibitory connections at

movement initiation which implies a potential role for the cerebellum in triggering the onset of voluntary movement.

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4.1

Cerebellar theta burst inhibition impairs eyeblink classical conditioning.

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B.P. van de Warrenburg, M.J. Edwards

Abstract

Theta burst stimulation (TBS) protocols of repetitive transcranial magnetic stimulation (rTMS) have after-effects on excitability of motor areas thought to be due to LTP- and LTD-like processes at cortical synapses. The present experiments ask whether, despite the low intensities of stimulation used and the anatomy of the posterior fossa, TBS can also influence the cerebellum. Acquisition and retention of eyeblink classical conditioning (EBCC) was examined in 30 healthy volunteers after continuous theta burst stimulation (cTBS) over the right cerebellar hemisphere. In subjects who received cerebellar cTBS, conditioned responses were fewer and their onsets were earlier (in the last half of the acquisition blocks) than those from control subjects. There was, however, no effect of cerebellar cTBS on the re-acquisition of EBCC in another session of EBCC 7–10 days later. There was also no effect of cerebellar cTBS on the re-acquisition of EBCC in subjects not naïve to EBCC when the stimulation was delivered immediately before a re-acquisition session. Control experiments verified that suppressive effects of cTBS on EBCC were not due to changes in motor cortical excitability or sensory disturbance caused by cTBS. Based on previous EBCC studies in various cerebellar pathologies, our data are compatible with the hypothesis that cerebellar cTBS has a focal cerebellar cortical effect, and are broadly in line with data from studies of EBCC in various animal models. These results confirm that cerebellar TBS has measurable effects on the function of the cerebellum, and indicate it is a useful non-invasive technique with which to explore cerebellar physiology and function in humans.

Introduction

Theta burst stimulation (TBS) is a protocol of repetitive transcranial magnetic stimulation (rTMS) where bursts of 50 Hz stimuli are given at a rate of 5 Hz [1]. This stimulation pattern was based on work in animal preparations demonstrating that direct electrical stimulation with a theta burst pattern of stimulation was more efficient in producing long-term depressive (LTD) and long-term potentiation (LTP) effects at stimulated synapses than regular repetitive stimulation [2]. TBS has significant advantages over other rTMS techniques in the brevity of the protocol (40–180 s) and the low intensities of stimulation used (typically 80% of active motor threshold) [1]. TBS has mainly been applied to study of motor areas, where assessment of its effects is relatively simple. Here, we wished to assess its possible effects on the cerebellum. One previous study using cerebellar TBS has reported effects on the excitability of the motor cortex and intracortical motor circuits [3], but in the absence of a more direct test of cerebellar function, the degree to which cerebellar TBS can truly influence cerebellar function remains unclear. Previous modelling studies of the effect of TMS show a decrease of 50% in induced current in tissue 10 mm away from the coil surface compared to tissue adjacent to the coil [4]. With the low intensities of stimulation used in TBS protocols and the anatomy of the posterior fossa, it seems quite possible that there would be insufficient penetration of current to have any effect on the cerebellum. This is of considerable experimental interest because it would be very beneficial to have a quick comfortable technique capable of inducing plastic changes in the cerebellum as a replacement for more lengthy and high-intensity protocols previously used [5-8].

Eyeblink classical conditioning (EBCC) is a protocol of associative motor learning in which paired presentation of a conditioned (CS) and unconditioned stimulus (US) leads to the production of a conditioned eyeblink response (CR). Studies using classical conditioning of the third eyelid, or nictitating membrane (NM), response of rabbits and EBCC in rodents and ferrets have revealed cerebellar circuitry underlying EBCC in which the cerebellar cortical Purkinje cell (PC) receives convergent afferent information about the CS and US via two separate pathways [9] with an additional potential convergence upon the underlying interpositus nucleus (IN) [10-12]. EBCC, with its heavy dependence on cerebellar function, is an ideal paradigm with which to assess and potentially quantify the possible influence of rTMS on the cerebellum. The wide variety of patient and animal studies that have assessed EBCC create an opportunity to contrast the effects of acute TBS-induced disruption of cerebellar function with those seen after cerebellar structural lesions.

In this study, we applied cTBS (continuous theta burst stimulation) over the right cerebellar hemisphere and measured its after-effects on acquisition and retention of EBCC.

Chapter 4.1

Methods

Subjects

Thirty volunteers (8 men and 22 women; mean age 29.38 ± 4.94; range: 22–44 years) participated in this study. All participants had no history of neurological, psychiatric or hearing disorders and did not take any medication acting on the central nervous system when studied. Informed consent was obtained from all participants and the study was approved by the local Ethics Committee and conducted in accordance with regulations laid down in the Declaration of Helsinki.

The majority of healthy volunteers were woman; however, no gender differences have previously been reported in literature in terms of effect of cTBS or EBCC.

Electromyographic recordings

Electromyographic (EMG) activity was recorded from both the orbicularis oculi (OO) and first dorsal interosseous (FDI) muscles using Ag–AgCl cup electrodes. OO EMG activity was recorded with the active electrode on the lower eyelid and the reference electrode approximately 3 cm distant on the lateral canthus [13]. FDI EMG activity was recorded with the active and the reference electrodes arranged in a classical belly-tendon montage. EMG raw signals were amplified and band-pass filtered (20 Hz to 3 kHz) using a Digitimer D360 amplifier (Digitimer Ltd, Welwyn Garden City, Herts, UK), digitized at a sampling rate of 5 kHz (CED 1401 laboratory interface; Cambridge Electronic Design, UK) and stored on a laboratory computer for on-line visual display. Data were analysed offline with dedicated software (SIGNAL software; Cambridge Electronic Design).

Eyeblink classical conditioning

The right supraorbital nerve was stimulated percutaneously through a pair of Ag–AgCl cup electrodes with the cathode over the supraorbital foramen and the anode 2 cm above. We used single, constant-current, square-wave electrical stimuli with a pulse width of 200 μs delivered through an electrical stimulator Digitimer DS7 (Digitimer Ltd). The electrical stimulus intensity was adjusted to obtain stable R2 responses (defined as reflex blink components at latency greater than 22 ms from stimulus onset). Typically, stimulus levels were 7 to 10 times the sensory threshold. This electrical supraorbital nerve stimulus was preceded by a tone (the CS) of 2 kHz and 400 ms duration produced by a tone generator (Grass Instruments, Quincy, MA, USA) and presented bilaterally to the subject via binaural headphones at an intensity 50–70 dB above the individual hearing threshold (minimal sound pressure level of 80 dB). CS intensities were kept identical across sessions for individual subjects. The CS inconsistently produced an acoustic startle response (alpha blink) occurring within 200 ms after CS onset. Repeated pairs of CS and US caused CRs to develop with onsets within 200 ms before US onset.

EBCC sessions consisted of seven blocks: six acquisition blocks followed by one within-session extinction block at the end of each session. The first nine trials of each EBCC induction

block consisted of nine CS–US pairs, the 10th trial was US only and trial 11 was CS only. The trials with CS only were given to verify that CRs were acquired independently of the US. The EBCC within-session extinction block consisted of 11 trials with only the CS. The inter-trial interval was randomized between 10 and 30 s.

Latencies to onset and peak of conditioned eyeblink responses were visually identified. CR onset was marked at the earliest point at which EMG activity began to rise from pre-CS EMG baseline level. In cases where the CR had multiple peaks, the amplitude and latency of peak amplitude were identified for largest amplitude peak.

CRs were defined as EMG activity lasting at least 50 ms or merging into superimposed UR of at least twice the amplitude of mean EMG baseline activity and clear rising slope [14]. We calculated CR amplitudes only where responses above baseline were detected, which is commonly referred to as 'CR magnitude'.

In subjects receiving neck cTBS or cerebellar cTBS, EBCC sessions started approximately 5 min after receiving rTMS. EBCC sessions lasted for approximately 25 min, a time frame over which plasticity effects of cTBS on motor cortex are active [1].

Transcranial magnetic stimulation

Single-pulse TMS was delivered through a monophasic Magstim 200 stimulator (Magstim Company Ltd, Whitland, Dyfeld, UK) connected to a figure-of-eight coil (external wing 9 cm in diameter) placed tangentially over the left primary motor cortex (M1) in the optimal position (hot spot) for eliciting motor-evoked potentials (MEPs) in the right FDI muscle. The right FDI hot spot (defined over the left M1 as the optimal position for eliciting MEPs in the right FDI muscle) was marked to ensure identical coil positioning throughout the experiment. Single-pulse TMS was delivered at the intensity able to evoke at baseline MEPs of ~1 mV peak-to-peak amplitude.

Repetitive TMS was delivered through a high-frequency biphasic magnetic stimulator (Magstim SuperRapid, Magstim) connected to a figure-of-eight coil (external wing 9 cm in diameter), placed tangentially over the right cerebellum with the handle pointing superiorly, 1 cm inferior and 3 cm right to the inion, a scalp position by former studies defined to predominantly target the superior and posterior lobules of the lateral cerebellum [3]. Repetitive TMS was delivered according to the cTBS protocol described by Huang et al. (2005) [1]. cTBS consisted of bursts of three pulses delivered at 50 Hz, repeated at intervals of 200 ms given in a continuous train lasting 40 s (600 pulses in total). The stimulation intensity of cTBS was set at 80% of active motor threshold (AMT). The AMT was defined as the lowest intensity evoking five MEPs of at least 200 µV in 10 consecutive trials while subjects maintained a low-level tonic contraction (20% of maximal voluntary contraction) in first dorsal interosseus and the coil was placed over the motor cortical 'hot-spot' for this muscle [15]. Sham rTMS was achieved by the delivery of cTBS with the same intensity as that used in the cerebellar stimulation but with the coil placed tangentially over the cervical muscles.

We recorded 30 MEPs from the right FDI at three time points: immediately before, 5 min after and 45 min after cerebellar cTBS and sham cTBS, to observe any influence cTBS might have on M1 excitability.

Experimental design

Subjects were studied while they were comfortably seated on a chair in a quiet room with normal indoor lighting. Two main experiments were performed to assess the effect of cerebellar cTBS on EBCC acquisition and retention. All participants were naïve to EBCC at the start of the study. The designs of experiments 1 and 2 are summarized in Figure 1.
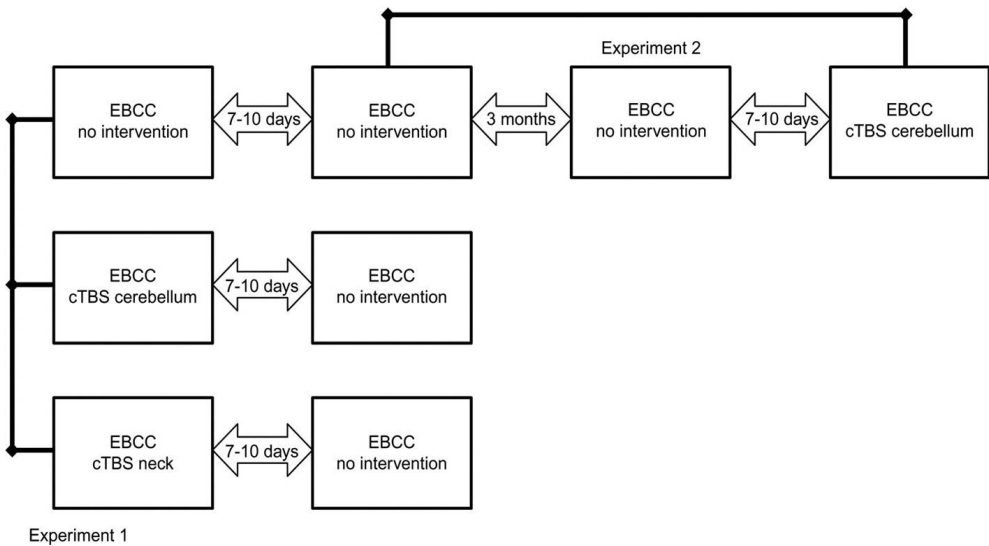
Figure 1 | Experimental designs of experiment 1 and experiment 2.

Experiment 1. In this experiment we assessed EBCC on two occasions separated by 7–10 days. The first group (no intervention, 12 participants) received no intervention in addition to these two sessions of EBCC. The second group (cTBS cerebellum, 10 participants) received cTBS over the cerebellum prior to the first session of EBCC, and no additional intervention prior to the second session of EBCC. The third group (cTBS neck, 8 participants) received cTBS over the neck muscles prior to the first session of EBCC, and no additional intervention prior to the second session of EBCC.

Experiment 2. Here we examined the effect of cerebellar cTBS on the re-acquisition of EBCC in participants who were not naïve to EBCC. We performed EBCC again in 7 of the 12 participants who had received EBCC without additional intervention in experiment 1. This EBCC session took place approximately 3 months after their last session of EBCC. Seven to ten days later, six of these participants had cTBS delivered over the cerebellum followed immediately by another session of EBCC.

Data analysis and statistics

Data were analysed using SPSS for Windows (version 16.0). The percentage of CRs, magnitude of CRs, the onset and peak latency of the CRs, the peak latency and magnitude of the URs, the number of alpha blinks and MEP peak-to-peak amplitude were used as dependent variables. Distribution of data was assessed using standard tests of normality (P value for the Shapiro–Wilk test of normality was 0.1; normality rejected). As the percentage of CRs over different blocks were not normally distributed these were analysed using non-parametric tests. We used repeated-measures ANOVA to compare magnitude and latencies of conditioned and unconditioned responses and number of alpha blinks. We also used repeated-measures ANOVA to assess whether cerebellar or neck cTBS affected the size of MEPs elicted from stimulation of the motor cortex. In all tests, the level of statistical significance was preset to P < 0.05. Unless otherwise stated all results are indicated as mean values ± the standard error of the mean (SEM). Bonferroni corrections were used in case of multiple comparisons.

Results

None of the subjects reported adverse effects related to the experimental procedures. The mean electrical threshold and electrical stimuli intensities used in the study sample were 1.69 ± 0.48 mA (range 0.60–3.0 mA) and 15.63 ± 4.80 mA (range 7.0–26.0 mA), respectively and were similar in all the experimental sessions ($F(7,53) = 0.48$; $P = 0.85$ and $F(7,51) = 0.2$; $P = 0.98$, respectively).

The AMT stimulator output in this study was 44.3 ± 6.24 (range 32–53) and did not differ between the three sessions: neck cTBS, cerebellar cTBS naïve participants and cerebellar cTBS second session ($F(2,23) = 0.72$; $P = 0.5$).

Experiment 1: EBCC acquisition and retention comparing no intervention, cerebellar cTBS and neck cTBS

Results of experiment 1 are visualized in Figure 2. A significant difference in mean percentage of CRs over the two consecutive sessions between the three different groups (no cTBS, cerebellar cTBS, neck cTBS) was disclosed using the Kruskal–Wallis test (H(2) = 7.023, $P = 0.030$). There was no difference in mean number of CRs between the neck cTBS and the no intervention groups ($Z = -0.85$; $P = 0.40$). No additional differences were present between these two groups in timing and magnitude of conditioned eyeblink responses, timing and magnitude of unconditioned eyeblink responses and number of alpha blinks during additional analysis. Neck cTBS and no intervention subjects were therefore combined as a single control group.

A significantly lower mean number of CRs over these two consecutive sessions was observed in the cerebellar cTBS group compared with the control group ($Z = -2.49$; $P = 0.013$).
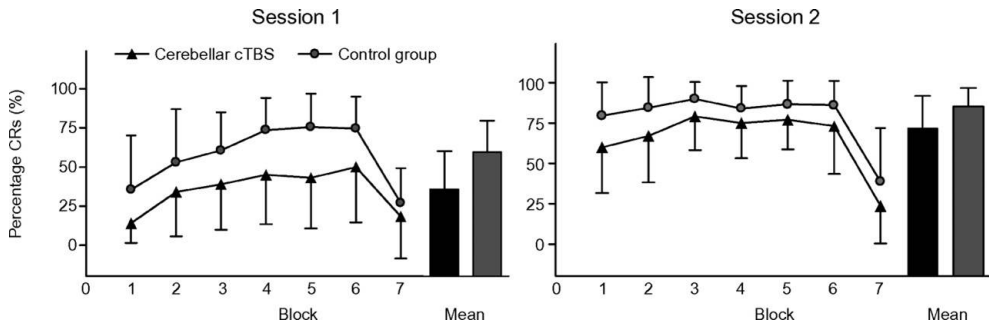
Figure 2 | Experiment 1 The percentages of conditioned responses in each block of testing (including block 7, the extinction block) are shown on the ***y*** axis. Data for the two groups of participants are plotted as black triangles (cerebellar cTBS) and shaded circles (control group). The figure shows data for the first session of EBCC and data for the second session of EBCC performed 7–10 days later. Mean percentage CR incidence over the six acquisition blocks is also shown to visualize overall performance. Error bars represent standard deviation.

A significant learning effect could be confirmed for both groups as the number of conditioned responses increased over the different sessions. A statistically significant effect for percentage of conditioned responses by BLOCK was confirmed using Friedman tests for the cerebellar cTBS and the control group in session 1 and session 2 (cerebellar cTBS group session 1: $X^2(6)$ = 18.09, *P* = 0.006; control group session 1: $X^2(6)$ = 24.31, *P* < 0.001; cerebellar cTBS group session 2: $X^2(6)$ = 60.99, *P* < 0.001; control group session 2: $X^2(6)$ = 44.52, *P* < 0.001). A significant higher number of mean responses for both groups in session 2 was additionally confirmed for the cerebellar cTBS (*Z* = −2.60, *P* = 0.009) and control group (*Z* = −3.81, *P* < 0.001) using Wilcoxon signed rank tests for mean percentage of conditioned responses between SESSION 1 and SESSION 2.

Level of retention of EBCC
As a measure of retention in session 2, the percentage of CRs in the last block of paired trials (block 6) in the first EBCC session was compared with the percentage of CRs in the first block of the second. Retention appeared normal for both groups as no significant difference between these two BLOCKS for either group (cerebellar cTBS: *Z* = −0.97, *P* = 0.33; control group: *Z* = −0.73, *P* = 0.46) could be confirmed using a Wilcoxon signed rank test.

Timing and magnitude of conditioned eyeblink responses
Onset and peak timing of conditioned responses are shown in Figure 3. A difference in timing of CRs was confirmed between the cerebellar cTBS group and control group for SESSION 1 but not SESSION 2. A repeated-measures ANOVA in the first conditioning session comparing the timing of both onset and peak latency of the conditioned eyeblink responses with main factors GROUP (control group, cerebellar cTBS) and BLOCK (blocks with paired trials) disclosed no significant effect for the between subjects factor GROUP ($F(1,19)$ = 0.13; *P* = 0.73), ($F(1,19)$ = 0.97; *P* = 0.34) or BLOCK ($F(5,95)$ = 1.71; *P* = 0.14), ($F(5,95)$ = 0.27; *P* = 0.94). A significant

GROUP × BLOCK interaction was observed for latency of peak ($F(5,95)$ = 2.37; P = 0.045) but not for onset of CRs ($F(5,95)$ = 2.13; P = 0.07).

We explored the effect of cTBS on timing of CRs. We only examined blocks 5 and 6 in this regard as the majority of CRs occurred in these blocks. We therefore performed two one-way ANOVAs for block 5 and block 6 with between subject factor GROUP (control group and cerebellar cTBS) that displayed significantly shorter CR peak latency for the cerebellar cTBS group in block 5 ($F(1,26)$ = 6.20; P = 0.020).
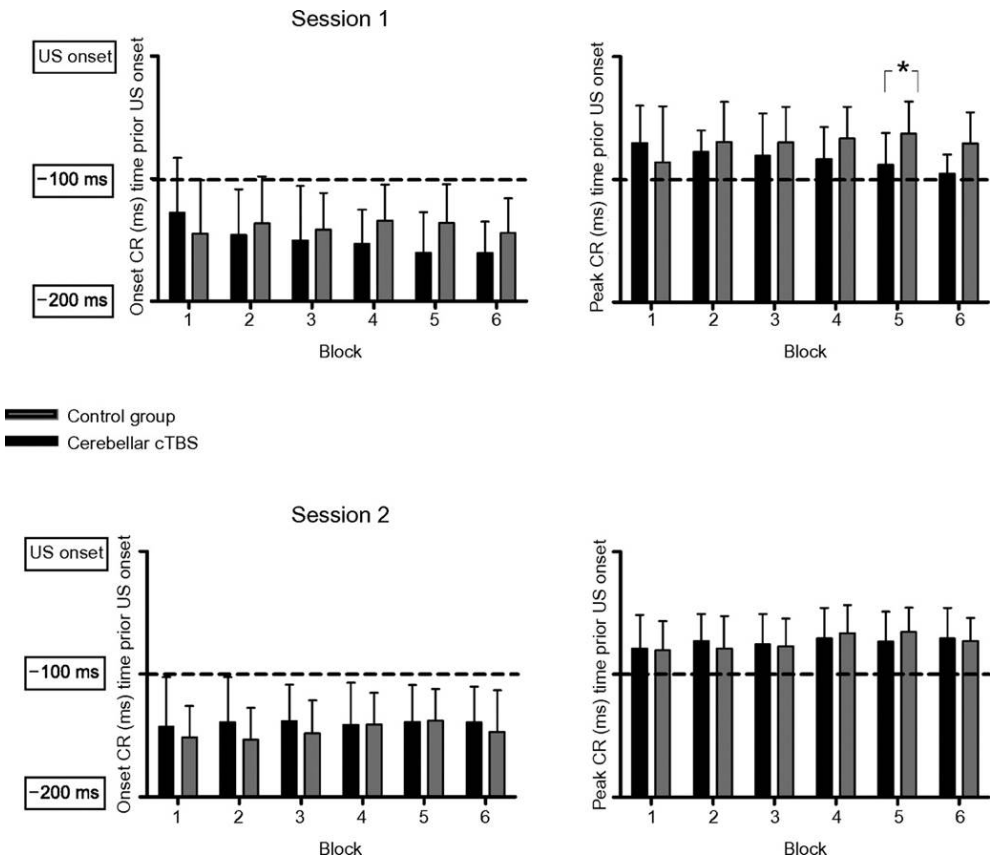
Figure 3 | Onset and peak timing of conditioned responses (CR) are shown on the y axis   Data for the two groups of participants are plotted for participants receiving cerebellar cTBS (black) and control group (grey) for both sessions. Error bars represent standard deviation. *Significant differences between groups (P < 0.05).

A repeated-measure ANOVA in the second conditioning session comparing the timing of both onset and peak latency of conditioned eyeblink responses with factor GROUP (control group, cerebellar cTBS) and BLOCK (6 blocks with paired trials) disclosed no significant effect for the between-factor GROUP ($F(1,25)$ = 0.46; P = 0.50), ($F(1,25)$< 0.001; P = 0.99) or BLOCK ($F(5,125)$ = 0.97; P = 0.44), ($F(5,125)$ = 1.98; P = 0.09) and no significant GROUP × BLOCK interaction ($F(5,125)$ = 0.82; P = 0.54), ($F(5,125)$ = 0.67; P = 0.65).

Two-way repeated-measures ANOVA for mean CR magnitude revealed no significant effect for the between-group factor GROUP (control group, cerebellar cTBS) ($F(1,24) = 1.68$; $P = 0.21$) nor for the within-group factor SESSION (first session, second session) ($F(1,24) = 3.63$; $P = 0.07$). The interaction GROUP × SESSION was also not significant ($F(1,24) < 0.001$; $P = 1$). Timing and amplitude of unconditioned responses and mean number of alpha blinks.

A general deficit in the performance of both learned and unlearned eyeblink responses was not present as timing and amplitude of URs and mean number of alpha blinks did not differ between the two groups. Two-way repeated-measures ANOVA for mean amplitude and mean peak latency of the URs disclosed no significant effects for the between-group factor GROUP (control group, cerebellar cTBS) ($F(1,28) = 2.32$; $P = 0.14$), ($F(1,24) = 0.56$; $P = 0.46$) and the within group factor SESSION (first session, second session) ($F(1,28) < 0.001$; $P = 1$), ($F(1,24) = 1.34$; $P = 0.26$). The interaction GROUP × SESSION was also not significant ($F(1,28) = 1.49$; $P = 0.23$), ($F(1,24) = 2.24$; $P = 0.15$).

Two-way repeated-measures ANOVA for mean number of alpha blinks revealed no significant effect for the between-group factor GROUP (control group, cerebellar cTBS) ($F(1,25) = 0.024$; $P = 0.88$) and the within-group factor SESSION (first session, second session) ($F(1,25) = 0.41$; $P = 0.53$). The interaction GROUP × SESSION was also not significant ($F(1,25) = 0.41$; $P = 0.53$).

Experiment 2: Retention of EBCC and response to cerebellar cTBS in subjects not naïve to EBCC

At 3 months following EBCC, participants appeared to have returned to baseline with respect to their response to a subsequent EBCC session. No significant retention of EBCC was seen in subjects receiving a further session of EBCC 3 months after their last session. The percentage of CRs in the first acquisition block of the EBCC session 3 months later was significantly lower compared with the last acquisition block (block 6) of their previous EBCC using a Wilcoxon signed rank tests between these two BLOCKs ($Z = −2.21$, $P = 0.027$). In addition no significant differences between the percentage of CRs over the complete EBCC session 3 months later and their first EBCC session were revealed using Mann–Whitney U tests (Acquisition Block 1 to 6: $Z = −0.97$, $P = 0.33$; $Z = −0.78$, $P = 0.44$; $Z = −0.59$, $P = 0.56$; $Z = −1.36$, $P = 0.18$; $Z = −0.78$, $P = 0.44$; $Z = −0.07$, $P = 0.95$; Extinction Block 7: $Z < 0.001$ $P = 1$).

cTBS over the cerebellum did not significantly change retention, re-acquisition and expression of EBCC when given 7–10 days following this session of EBCC.

Figure 4 reveals a trend towards overall lower percentages of CRs for a second EBCC session with cerebellar cTBS (experiment 2, session 2) from the second EBCC session of the no intervention group from experiment 1, but statistical analysis using Mann–Whitney U tests did not disclose any significant differences between the different BLOCKS (Acquisition Block 1 to 6: $Z = −1.22$, $P = 0.22$; $Z = −0.16$, $P = 0.87$; $Z = −1.53$, $P = 0.13$; $Z = −0.98$, $P = 0.33$; $Z = −1.27$, $P = 0.20$; $Z = −1.15$ $P = 0.94$; Extinction Block 7: $Z = −0.081$ $P = 0.94$).

The percentage of CRs in the last acquisition block of the fourth EBCC session and the first block of the EBCC session 7–10 days previously were comparable as well. We found no significant difference between these two BLOCKS for either group ($Z = -0.95$, $P = 0.34$) using a Wilcoxon signed rank test.
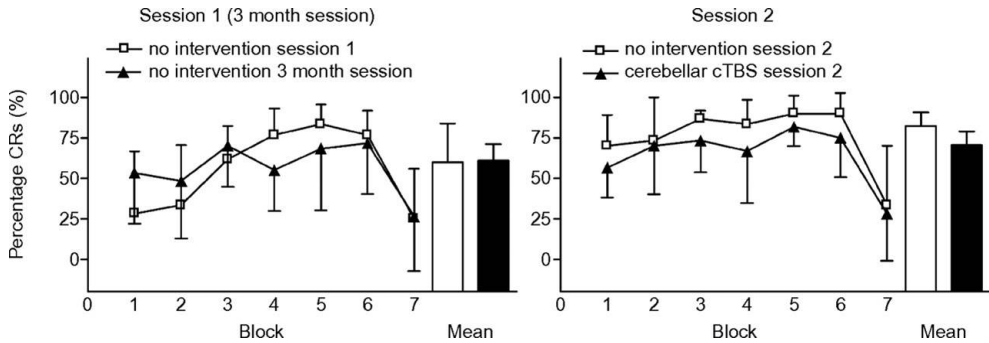
Figure 4 | The percentages of conditioned responses in each block of testing (including block 7, the extinction block) are shown on the y axis. The left side illustrates participants first session of EBCC (open squares) and their third session of EBCC performed at least 3 months following their last session of EBCC. On the right, data from a fourth session of EBCC before which participants received cTBS (filled triangles) and for comparison, data from their second session of EBCC given without cTBS in experiment 1 is illustrated (open squares). Mean percentage CR incidence over the six acquisition blocks is also shown to visualize overall performance. Error bars represent standard deviation.

Timing and magnitude of conditioned eyeblink responses

No significant differences in timing and magnitude of CRs were found between the second EBCC session of the no cTBS group from experiment 1 and the second session from experiment 2 where subjects who had received an EBCC session 7–10 days previously received cTBS before a final EBCC session.

A repeated-measures ANOVA was conducted to compare changes in timing of conditioned eyeblink responses over conditioning blocks with factors GROUP (no intervention, cerebellar cTBS) and BLOCK (6 blocks with paired trials). No significant effect for the between-subjects factor GROUP ($F(1,9) = 3.28$; $P = 0.10$), ($F(1,9) = 2.47$; $P = 0.15$) or BLOCK ($F(5,45) = 2.07$; $P = 0.087$), ($F(5,45) = 1.83$; $P = 0.13$) and no significant GROUP × BLOCK interaction ($F(5,45) = 0.35$; $P = 0.88$), ($F(5,45) = 1.03$; $P = 0.41$) was observed for both onset and peak latency of conditioned responses.

A one-way ANOVA comparing mean magnitude of CRs with between-subject factor GROUP (cTBS and no intervention) disclosed no significant difference in magnitude of conditioned responses between these two groups ($F(1,11) = 3.08$; $P = 0.11$).

Effects of cerebellar and sham cTBS on the MEP peak-to-peak amplitude

In order to assess the effect of cerebellar and neck cTBS on motor cortical excitability we performed a two-way ANOVA with STIMULATION (cerebellar cTBS, neck cTBS) and TIME (before cTBS, 5 min post cTBS, 45 min post cTBS) as main factors. There was no significant effect for STIMULATION ($F(1,17)$ = 3.68; P = 0.072), TIME ($F(2,34)$ = 0.023; P = 0.98) and no STIMULATION × TIME interaction ($F(2,34)$ = 0.067; P = 0.94).

Discussion

The present results show that cTBS delivered over the cerebellar hemisphere had measurable after-effects on EBCC. When applied to naïve subjects before their first session of EBCC, it impaired the acquisition and timing of CRs but did not affect retention when EBCC was tested 1 week later. If cTBS was applied immediately before a second EBCC session, it had no effect on re-acquisition of EBCC. As reported by others, there was good retention of EBCC in subjects after 7–10 days that was not present at 3 months [16].

Our results strongly suggest that cTBS can interfere with cerebellar function. Theta burst stimulation can induce lasting changes in corticospinal excitability thought to involve LTP-/LTD-like effects on cortical synapses. The pattern of delivery of TBS determines the direction of change and cTBS results in an LTD-like effect. Different animal studies have investigated the role of LTD in cerebellar motor learning by studying different types of LTD expression-deficient mutant mice. Most of these studies found general impairments in motor learning [17-19]. However, a recent study, with a mouse model thought to affect LTD in a more specific way, did not reproduce this impairment and suggested that previous models did not only affect LTD at the PC level but also affected other forms of cerebellar plasticity [20]. In our study, cTBS is likely to only directly stimulate the cerebellar cortex, as it would be unlikely that the intensities used with cTBS could directly affect the deep cerebellar nuclei. However, this does not exclude an effect on deep cerebellar structures (e.g. deep cerebellar nuclei) or extracerebellar structures (e.g. olivary nuclei) as remote secondary effects can occur secondary to rTMS [21].

How do our results fit with previous studies of EBCC in patients with cerebellar lesions? The majority of studies in patients show that unilateral cerebellar lesions cause unilateral reduction of EBCC [10, 22-24]. Gerwig et al. (2003) examined EBCC in patients with lesions of the superior cerebellar artery (SCA) territory (the SCA supplies hemisphere lobule VI and IN) and found that although unilateral lesions impaired CR acquisition there was no difference between the effect of pure cortical lesions and lesions that also affected deep cerebellar nuclei [14]. In contrast, Woodruff-Pak et al. (1996) observed greater impairment of acquisition if lesions involved the cerebellar nuclei as well as the cortex [24]. Gerwig et al. (2010) recently compared multiple EBCC sessions between patients with degenerative cerebellar disorders affecting cortex and patients with focal cortical lesions of lobules VI and/or Crus I [16]. Acquisition deficits were less marked in patients with focal cortical lesions. Patients with focal cortical lesions were able to retain conditioned responses when tested in consecutive

sessions but showed no further improvement in these additional EBCC sessions. Patients with degenerative cerebellar disorders did not acquire, retain or improve conditioned responses over repeated sessions, in accordance with previous results of Timmann et al. (2005) [25].

The clinical evidence above is limited because of the lack of uniform lesion localization and the confounding factors of compensatory change following chronic cerebellar lesions [26]. However, they do support the hypothesis that cTBS in healthy subjects predominantly targets the cerebellar cortex as the EBCC deficits in our cTBS subjects are similar to those seen in focal cerebellar cortical lesions, in which acquisition is reduced and retention retained, rather than the picture in degenerative cerebellar disorders where there is a loss of acquisition and retention.

As further support for this hypothesis, cerebellar cortical lesions have also been reported to affect the timing of CRs, particularly if they affect the anterior lobe [27, 28]. Gerwig et al. (2010) evaluated delay eyeblink conditioning over multiple sessions in patients with focal cerebellar cortical lesions including lobules VI and Crus I [16]. Only few lesions in this study extended into the anterior lobe and in these cases only a small area of the anterior lobe was lesioned. Patients with focal cortical lesions nevertheless exhibited earlier timing of CRs in their first conditioning session but improved timing close to control values over the two subsequent conditioning sessions. Similarly, in the experiments reported here cTBS produced a subtle shortening of the CR latency in the last half of the conditioning blocks.

Overall, cTBS was more effective in disrupting the acquisition of EBCC compared with the retention and re-acquisition of EBCC. This may reflect the general finding that acquisition is often more easily disturbed than retention by a range of interventions, and that overtraining can make retention particularly resistant (for example, Harvey et al. 1993 [29]). However, there are other possibilities: longer-term storage of memory for EBCC might be more dependent upon extracerebellar circuits. Alternatively, if the main effect of cTBS is upon the cerebellar cortex, then the findings are consistent with the suggestion that cerebellar cortex is more involved in the acquisition of EBCC than in the retention of EBCC. There is continued debate about the roles of the cerebellar cortex and nuclei in the acquisition and retention of cerebellum-dependent forms of motor learning in general and EBCC in particular [11, 30-32]. We accept (see below) that our results with regard to the effect of cTBS on retention of EBCC need to be treated with caution given the low subject numbers in this part of the experiment. However, our results in humans are broadly in line with previous animal and human studies and, like the majority of them, do not clearly dissociate the roles of cerebellar cortex and nuclei in the acquisition and retention of EBCC. The lack of significant effect of cTBS on retention of EBCC in a subsequent session may be due to the memory trace being either localized in the deep cerebellar nuclei which are not affected by cTBS or outside the cerebellum. Alternatively, the memory trace may substantially involve the cerebellar cortex but be more resistant to the effects of cTBS than the mechanisms involved in acquisition. Nonetheless, despite these limitations, our study demonstrates for the first time reversible inhibition of EBCC in humans.

Chapter 4.1

We had only a small number of subjects for the second experiment assessing the effect of cTBS given prior to a re-acquisition EBCC session. We found no effect of the stimulation on number of CRs recorded in the subsequent EBCC session, but we cannot exclude the possibility that we have missed a small effect due to insufficient numbers of subjects. The subjects taking part in this part of the experiment received four sessions of EBCC over a 4 month period, and therefore it was difficult to secure sufficient participants. We therefore accept that the conclusions we can draw from these data must be tentative. We have not, in this study, fully explored the temporal profile of the interaction between EBCC and cTBS. Further experiments in humans or in animal models may be appropriate to explore such interactions. As cTBS over the neck muscles had no effect on EBCC, we suggest that the effects of cerebellar rTMS cannot be explained by distraction effects due to the sensory stimulation. A study by Koch et al. (2008) reported that cerebellar cTBS decreases motor cortical excitability; no significant difference in motor cortex excitability was, however, observed after cerebellar cTBS in a recent study by Popa et al. (2010) [3, 33]. We looked at changes in MEP size before applying cTBS, 1 min after cTBS and after finishing the conditioning session (approximately 40 min after cTBS) but did not observe any reduction in MEP size. Methodological differences between our experiments and those of Koch et al. (2008) such as estimation of active motor threshold, could account for these different findings on the effect of cerebellar cTBS on motor cortical excitability [3].

Conclusions

The fact that cerebellar TBS has clear effects on EBCC in humans is strong evidence that cTBS can influence cerebellar function. It suggests that despite the low intensities of stimulation used and the anatomical constraints of the posterior fossa, cTBS can stimulate cerebellar cortex with measurable effects on behaviour. This simple, quick and comfortable rTMS protocol has advantages over traditional rTMS protocols, and the results with regard to EBCC demonstrate the potential usefulness of this technique in studying cerebellar physiology non-invasively in humans.

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4.2

Cerebellum-dependent associative learning deficits in primary dystonia are normalized by rTMS and practise.

Abstract

Eyeblink classical conditioning (EBCC) is a cerebellum-dependent paradigm of associative motor learning, and abnormal EBCC is a neurophysiological indicator of cerebellar dysfunction. We have previously demonstrated impaired EBCC in patients with primary dystonia, but it remains uncertain if this represents actual cerebellar pathology or reflects a functional cerebellar disruption. We examined this further by: (1) studying acquisition and retention of EBCC in a second session in eight patients with cervical dystonia (CD) who had a first session 7–10 days earlier; and (2) by investigating the potential of continuous theta burst stimulation (cTBS) over the right cerebellar hemisphere to modify a first-ever EBCC session in 11 patients with CD. EBCC data of eight healthy controls previously studied were used for additional between-group comparisons. We observed an improvement of EBCC in a second session in patients with CD, which is in contrast to patients with proven cerebellar pathology who do not show further improvement of EBCC in additional sessions. We also found that cerebellar cTBS paradoxically normalized EBCC in patients with CD, while we previously showed that it disrupts EBCC in healthy volunteers. Combined, these two experiments are in keeping with a functional and reversible disruption of the cerebellum in dystonia, a phenomenon that is probably secondary to either cerebellar compensation or to cerebellar recruitment in the abnormal sensorimotor network.

Introduction

There is a growing body of evidence that suggests a role of the cerebellum in the pathophysiology of dystonia. The unanswered question is whether cerebellar dysfunction in dystonia is based on actual cerebellar (structural) pathology or results from functional cerebellar disruption. The former is supported by cerebellar grey matter abnormalities [1-3] and microstructural deficits in white matter integrity [4, 5] on neuroimaging; the latter by, for example, increased cerebellar metabolic activity in patients with dystonia and non-manifesting DYT1 carriers[6-11].

Eyeblink classical conditioning (EBCC) is a form of associative motor learning where paired presentation of a conditioned (CS; auditory tone) and unconditioned stimulus (US; electrical stimulus or air puff) results in a conditioned eyeblink response (CR; Figure 1). The cerebellar cortex, cerebellar nuclei and inferior olives are critical neuroanatomical sites for the acquisition and retention of CRs[12-14]. Cerebellar lesions and cerebellar degeneration both cause impairments in EBCC that are not improved by repeated sessions of conditioning. Patients with adult-onset focal dystonia also have abnormal EBCC[15]. However, it is not known whether this impairment is similar to what is seen in cerebellar pathologies, and also how this finding relates to cerebellar hypermetabolism seen on functional imaging in dystonia.
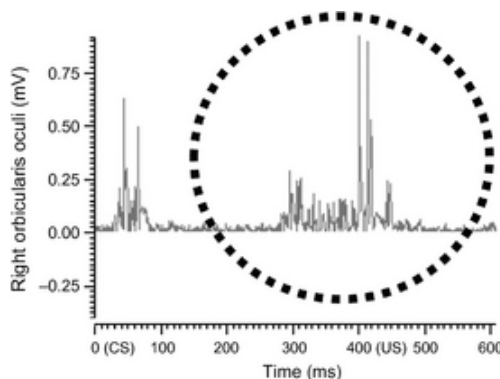
Figure 1 | Screenshot of EMG recording over the right orbicularis oculi (OO) muscle during EBCC. The CS (auditory tone) produces an acoustic startle response (alpha blink). A conditioned response (CR) is visible before the onset of the US (supraorbital nerve stimulus).

Here, we further explored this EBCC deficit in primary dystonia, and in particular its potential for modification in two different experiments (Figure 2). We first studied EBCC in two consecutive sessions to determine whether an improvement of EBCC over subsequent sessions occurred in patients with dystonia. Improvement of EBCC could be observed by a higher percentage of CRs during an EBCC session or seen as improved timing of CRs, i.e. CRs occurring later and closer to US onset. While healthy controls indeed have the capacity to improve EBCC

upon a further session, this is not expected in structural cerebellar pathologies[16-19]. This experiment would therefore allow us to comment on whether the EBCC defect in dystonia could indeed be due to a more structural type of cerebellar dysfunction. Second, we targeted the cerebellum with an inhibitory continuous theta burst stimulation (cTBS) prior to a first-ever EBCC session. In healthy subjects, this impairs EBCC[20], but we wondered what the effect would be in dystonia, given the cerebellar hyperactivity that is suggested by various imaging studies. Other transcranial magnetic stimulation (TMS) studies in dystonia have shown that normalization of hypermetabolism with an inhibitory form of repetitive (r)TMS can improve task performance, for example writing in focal hand dystonia after inhibitory rTMS over the premotor cortex. This second experiment would clarify the possibility of a more functional, and perhaps reversible, type of cerebellar dysfunction in dystonia.
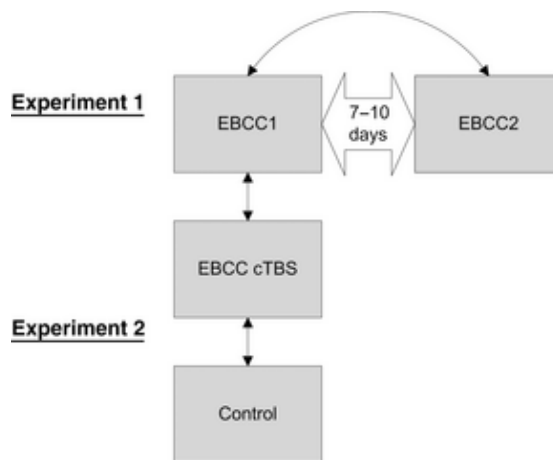
Figure 2 | Experimental design. The following abbreviations are used: EBCC [first eyeblink classical conditioning (EBCC) session in patients with CD naive to EBCC]; EBCC 2 (second EBCC session in patients with CD); EBCC cTBS [first EBCC session in patients with CD naive to EBCC with cerebellar continuous theta burst stimulation (cTBS) prior to EBCC]; Control (first EBCC session in healthy controls naïve to EBCC).

Methods

Nineteen patients with primary cervical dystonia (CD) participated in this study. Patients taking neurotropic medication were excluded. Informed consent was obtained from all participants, and the study was approved by the local Ethics Committee and conducted in accordance with regulations laid down in the Declaration of Helsinki. Eight historical control subjects were selected from our previous study in which identical methods to assess EBCC were used[15].

Study design

Two experiments were performed to examine EBCC in patients with CD. In the first experiment, we assessed EBCC in eight patients on two occasions separated by 7–10 days

to examine acquisition deficits and retention. In the second experiment, 11 other patients with CD received cerebellar cTBS prior to a first-ever EBCC session to examine the effect of this inhibitory form of rTMS on EBCC (Figure 2). The reason for using a second group of patients with CD, rather than doing both experiments in one group, is the long duration of EBCC retention.

Electromyographic (EMG) recordings

EMG activity was recorded from both the orbicularis oculi (OO) and first dorsal interosseous (FDI) muscles using silver electrodes with a silver chloride coating. OO activity was recorded with the active electrode on the lower eyelid and the reference electrode approximately at 3 cm distance on the lateral canthus [21]. FDI activity was recorded with the active and the reference electrodes arranged in a classical belly-tendon montage. EMG raw signals were amplified and band-pass filtered (20 Hz–3 kHz) using a Digitimer D360 amplifier (Digitimer, UK), digitized at a sampling rate of 5 kHz (CED 1401 laboratory interface; Cambridge Electronic Design, UK) and stored on a laboratory computer for online visual display. Data were analysed offline with dedicated software (SIGNAL software; Cambridge Electronic Design).

EBCC

The right supraorbital nerve was stimulated percutaneously through a pair of Ag/AgCl cup electrodes with the cathode over the supraorbital foramen and the anode 2 cm above (the US). We used single, constant-current, square-wave electrical stimuli with a pulse width of 200 μs delivered through an electrical stimulator Digitimer DS7 (Digitimer, UK). The electrical stimulus intensity was adjusted to obtain stable R2 responses (defined as reflex blink components at latencies above 22 ms from stimulus onset).

Typically, stimulus levels were 7–9 × sensory threshold. This electrical supraorbital nerve stimulus was preceded by a tone (the CS) of 2 kHz and 400 ms duration produced by a tone generator (Grass Instruments, Quincy, MA, USA) and presented bilaterally via binaural headphones at an intensity 50–70 dB above the individual hearing threshold (minimal sound pressure level of 80 dB). CS intensities were kept identical across sessions for individual subjects. The CS inconsistently produced an acoustic startle response (alpha blink) occurring within 200 ms after CS onset. Repeated pairs of CS and US caused CRs to develop with onsets within 200 ms before US onset (Figure 1).

EBCC sessions consisted of seven blocks: six acquisition blocks followed by one within-session extinction block at the end of each session. The first nine trials of each EBCC induction block consisted of nine CS–US pairs, the 10th trial was US only, and trial 11 was CS only. The trials with CS only were given to verify that CRs were acquired independently of the US. The EBCC extinction block consisted of 11 trials with only the CS. The inter-trial interval was randomized between 30 and 40 s.

CRs were defined as EMG activity lasting at least 50 ms or merging into superimposed unconditioned response (UR) of at least twice the amplitude of mean EMG baseline activity

and clear rising slope [22]. We calculated CR amplitudes only when responses above baseline were detected, which is commonly referred to as 'CR magnitude'. Latencies to onset and peak of CRs were visually identified. In cases of multiple CR peaks, the amplitude and latency of the largest amplitude peak was used.

TMS

Single-pulse TMS was delivered through a monophasic Magstim 200 stimulator (The Magstim Company, UK) connected to a figure-of-eight coil (external wing 9 cm in diameter) placed tangentially over the left M1 in the optimal position (hot spot) for eliciting motor-evoked potentials (MEPs) in the right FDI muscle. The right FDI hotspot (defined as the optimal position over the left M1 for eliciting MEPs in the right FDI muscle) was marked to ensure identical coil positioning throughout the experiment. Single-pulse TMS was delivered at the intensity able to evoke at baseline MEPs of ~1 mV peak-to-peak amplitude.

rTMS was delivered through a high-frequency biphasic magnetic stimulator (MagstimSuperRapid; The Magstim Company) connected to a figure-of-eight coil (external wing 9 cm in diameter), placed tangentially over the right cerebellum with the handle pointing superiorly, 1 cm inferior and 3 cm right to the inion, a scalp position defined by former studies to predominantly target the superior and posterior lobules of the lateral cerebellum. rTMS was delivered according to the protocol used by Huang et al. (2005). cTBS consisted of bursts of three pulses delivered at 50 Hz, repeated at intervals of 200 ms given in a continuous train lasting 40 s (600 pulses in total). The stimulation intensity of cTBS was set at 80% of active motor threshold (AMT).

We recorded 30 MEPs from the right FDI at three time points: immediately before, 5 min after, and 45 min after cerebellar cTBS, to observe any influence that cTBS might have on M1 excitability. In subjects receiving cerebellar cTBS, EBCC sessions started approximately 5 min after receiving rTMS. EBCC sessions lasted for approximately 25 min, a time frame over which plasticity effects of cTBS on the motor cortex are detectable [23].

Analysis

Data were analysed using SPSS for Windows (version 16.0). Age, proportion of male subjects, Toronto Western Spasmodic Torticollis Scale (TWSTRS) score and disease duration were used to compare the different groups. The percentage of CRs, the onset and peak latency of the CRs, the peak latency and magnitude of the URs, the number of alpha blinks, and MEP peak-to-peak amplitude were used as dependent variables for EBCC analysis. The distribution of data was assessed using standard tests of normality (P-value for the Shapiro–Wilks test of normality was 0.1; normality rejected). As the percentage of CRs over different blocks was not normally distributed, these were analysed using non-parametric tests. In all tests, the level of statistical significance was preset to $P < 0.05$. Unless otherwise stated, all results are indicated as mean values ± standard error of the mean (SEM).

Results

Subject demographics are shown in Table 1. There were no significant differences between the three subject groups in terms of age ($F_{2,22}$ = 0.28, P = 0.76) or proportion of male subjects (χ^2_2 = 3.23, P = 0.20). Between the two patient groups, no differences were found for disease severity (TWSTRS score; t_{16} = 1.94, P = 0.99) or duration (t_{16} = −0.029, P = 0.98). None of the subjects reported side-effects related to the experimental procedures. The mean electrical threshold and electrical stimuli intensities used in the study sample were 1.69 = 0.48 (range 0.60–3.0 mA) and 15.63 = 4.80 (range 7.0–26.0 mA), respectively, and were similar in all the experimental sessions ($F_{7,53}$ = 0.48, P = 0.85; and $F_{7,51}$ = 0.2, P = 0.98, respectively).

Table 1 | Subject characteristics

	Number	Male subjects	Age (years)	TWSTRS score	Disease duration (years)
CD second EBCC session	8	2	62.63 ± 6.72	32.50 ± 11.93	13 ± 6.97
CD cerebellar cTBS	11	2	60.20 ± 8.04	22.55 ± 9.88	13.1 ± 7.70
Controls no intervention	8	3	57.59 ± 20.11	–	–

CD, cervical dystonia; cTBS, continuous theta burst stimulation; EBCC, eyeblink classical conditioning; TWSTRS, Toronto Western Spasmodic Torticollis Scale.

Percentage of eyeblink CRs over the first session

When using a Mann–Whitney *U*-test to compare the mean percentage of CRs of patients with CD with eight age-matched healthy controls, a significant lower mean percentage of CRs was observed in a first EBCC session of patients with CD studied without intervention (Z = −2.00, P = 0.045), thereby confirming former findings of EBCC impairments in primary dystonia (Teo et al., 2009).

Experiment 1

Change in percentage of CRs between sessions

A significant effect for mean percentage of CRs depending on SESSION was confirmed for the no-intervention group (Z = −2.20, P = 0.028) using a Wilcoxon Signed Rank test. This indicates a higher number of mean CRs in session 2 compared with session 1. Patients with CD are therefore capable of increasing their number of CRs over repeated EBCC sessions (Figure 3).

Timing of and magnitude of CRs between sessions

Because of the low number of CRs, a comparison for mean onset and peak latency was made for CRs occurring in block 6 between sessions 1 and 2 using a paired sample *t*-test.
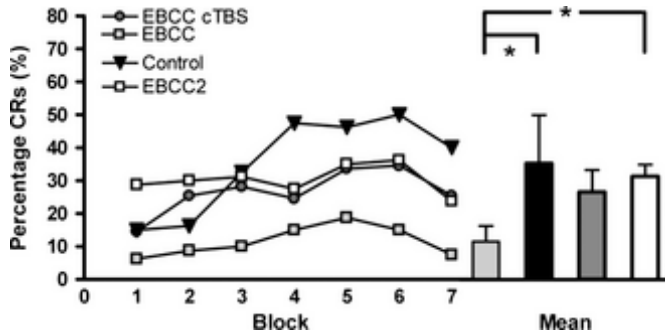
Figure 3 | The percentages of conditioned responses (CRs) in each block of testing (including block 7, the extinction block) are shown on the *y*-axis. Data for the different groups of participants are plotted. The following abbreviations are used: EBCC [first eyeblink classical conditioning (EBCC) session in patients with CD naive to EBCC]; EBCC 2 (second EBCC session in patients with CD); EBCC cTBS [first EBCC session in patients with CD naive to EBCC with cerebellar continuous theta burst stimulation (cTBS) prior to EBCC]; Control (first EBCC session in healthy controls naïve to EBCC). Mean percentage CR incidence over the six acquisition blocks is also shown to visualize overall performance. Error bars represent standard deviation. Asterisks indicate significant differences between groups ($P < 0.05$).

No significant differences were observed for either onset ($t_3 = 1.14$, $P = 0.34$) or peak timing ($t_3 = -0.14$, $P = 0.90$) of CRs. Latencies of CRs were therefore not different between these two sessions. A paired sample *t*-test for mean amplitude of the CRs disclosed no differences between the two sessions ($t_5 = -0.06$, $P = 0.95$).

Timing and magnitude of URs and mean number of alpha blinks

A paired sample *t*-test for mean amplitude and mean peak latency of the URs disclosed no differences for either mean amplitude ($t_5 = 0.23$, $P = 0.83$) or mean latency of URs ($t_5 = 0.81$, $P = 0.46$) between the two sessions. A paired sample *t*-test for mean number of alpha blinks also revealed no significant difference ($t_1 = 1.15$, $P = 0.46$). This indicates that improved EBCC was selective to CRs and could not be explained by a general effect, as timing and amplitude of URs and mean number of alpha blinks did not differ between the two groups.

Dystonia characteristics and mean percentage of CRs

No correlations between mean percentage of CRs over subsequent sessions and TWSTRS score or disease duration were observed using Pearson correlations.

Experiment 2

Percentage of CRs

When using a Mann–Whitney *U*-test to compare the mean percentage of CRs with eight age-matched healthy controls, a lower mean percentage of CRs was observed in patients with CD studied without intervention ($Z = -2.00$, $P = 0.045$), while there was no difference between

healthy controls and patients with CD who received cTBS prior to a first EBCC session ($Z = -0.79$, $P = 0.4$; Figure 3).

Direct comparisons of the dystonia groups using a Mann–Whitney U-test for mean percentage of CR did not yield a significant difference between the two groups ($Z = -1.29$, $P = 0.20$). Only when excluding so-called 'non-responders' (patients who did not acquire any CR) from both groups (two from no-intervention group and three from cerebellar cTBS group), was a significant difference for mean percentage of CRs observed employing a Mann–Whitney U-test, indicating a higher percentage of CRs in the cerebellar cTBS group for patients acquiring CRs ($Z = -2.13$, $P = 0.03$).

However, Friedman tests confirmed a significant effect for percentage of CRs by acquisition BLOCK (blocks 1–6) for the cerebellar cTBS group, but not for the no-intervention group (cerebellar cTBS group session 1: $\chi^2_5 = 12.87$, $P = 0.03$; no-intervention group session 1: $\chi^2_5 = 9.27$, $P = 0.10$). This indicates that only the cerebellar cTBS group increased their number of CRs during the acquisition blocks of this session. Together, these results suggest strongly that cTBS improves EBCC deficits in CD.

Timing and magnitude of CRs

A comparison for mean onset and peak latency was made for CRs in block 6 of the first EBCC session between these two dystonia groups. No difference was observed for either onset ($F_{1,10} = 0.12$, $P = 0.73$) or peak timing ($F_{1,10} = 0.20$, $P = 0.66$) of CRs. Latencies of CRs were therefore not different between these two groups. Figure 4 suggests that patients adapted timings of CRs, that is CRs occurred later and closer to US onset, but analyses of timing of CRs over subsequent blocks yielded no changes for either group. A paired sample t-test for mean magnitude of the CRs disclosed no differences between the two groups ($t_{13} = 0.61$, $P = 0.51$).
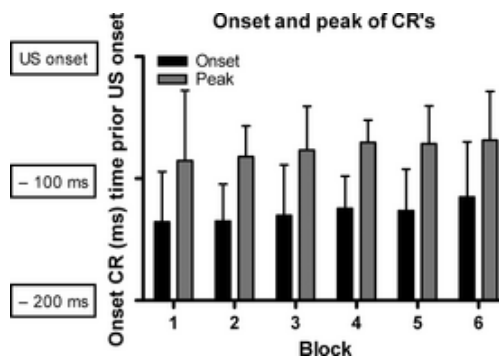
Figure 4 | Timing of conditioned responses (CRs). Onset and peak of CRs of patients with CD (studied with and without intervention) in milliseconds (ms) prior to unconditioned stimulus (US) onset during a first EBCC session.

Timing and amplitude of URs and mean number of alpha blinks

One-way anova for mean amplitude and mean peak latency of the URs disclosed no differences for either mean amplitude ($F_{1,14}$ = 0.41, P = 0.53) or mean latency of URs ($F_{1,14}$ = 0.57, P = 0.47) between the two groups. One-way anova for the mean number of alpha blinks revealed no difference ($F_{1,7}$ = 0.38, P = 0.56). This indicates that effects of cTBS on normalization of EBCC in primary dystonia EBCC were selective to CRs, as timing and amplitude of URs and mean number of alpha blinks did not differ between the two groups.

Effect of cerebellar cTBS on MEP peak-to-peak amplitude

A repeated-measurements anova displayed no effect of TIME (before cTBS, 5 min post-cTBS, 45 min post-cTBS) on MEP amplitude ($F_{2,18}$ = 1.62, P = 0.23).

Dystonia characteristics and mean percentage of CRs

No correlations between the mean number of CRs during session 1 and TWSTRS score or disease duration were observed using Pearson correlations.

Discussion

Animal studies have shown that the cerebellum is the critical neuroanatomical site for acquisition, timing and retention of EBCC responses. Here, we explored the known EBCC deficit in primary dystonia, and observed that EBCC in dystonia could be modified by practice (via repeated sessions of EBCC) and by direct non-invasive modulation of cerebellar excitability (through inhibitory cTBS).

Our data from the first experiment, the two consecutive EBCC sessions, therefore argue against major structural cerebellar changes in dystonia. Patients with cerebellar degeneration as well as patients with focal cerebellar lesions (even when restricted to the cerebellar cortex) have permanent deficits in the acquisition of EBCC responses [16-19]. Such patients might acquire CRs in a first conditioning block and normal retention might be seen in a subsequent session, but no further increase of CRs is observed in a second or third EBCC session. Timing of the CR is also cerebellum-dependent and timing deficits in the CR are seen in these patients, particularly in those with cerebellar degeneration [18]. We here show that patients with CD have, in contrast, the capacity to improve their number of CRs in a subsequent EBCC session, even when the interval between these two sessions is 7–10 days. In addition to this, no timing deficits were observed.

Our second experiment of a first-ever EBCC session following cerebellar TBS further supports a functional and modifiable involvement of the cerebellum in dystonia. Previously, we examined acquisition and retention of EBCC in 28 healthy volunteers following cTBS over the right cerebellar hemisphere [20], and found that cerebellar cTBS led to an impairment of EBCC. In the current study, applying the same technique to patients with CD, we found an 'improvement' of EBCC as patients developed EBCC profiles similar to age-matched controls. A general improvement in the performance of both learned and unlearned eyeblink

responses does not explain our findings, because timing and amplitude of URs and mean number of alpha blinks did not differ between the two groups.

In our previous study [20], we did not observe any effect of cerebellar cTBS on the re-acquisition of EBCC in another session of EBCC 7–10 days later; there was also no effect of cerebellar cTBS on the re-acquisition of EBCC in subjects not naïve to EBCC when the stimulation was delivered immediately before a re-acquisition session. Because of these earlier observations, we did not include, in the current study, a second session in which we studied whether cerebellar cTBS in patients might synergize the effect of practice. But when we compare the results from our previous study with our current findings, a ceiling effect might be present in healthy controls, which is not present in patients. The healthy controls in our first study are, however, not age-matched to our current patient group and we therefore refrained from direct statistical comparisons. But when we examine the percentage increase of mean number of CRs in a second EBCC session in subjects studied without intervention and in patients, a higher percentage of increase can be found in patients vs controls in the second session (477% increase vs. 177% increase). This underlines the effect of practice in patients and/or the possibility of a ceiling effect in healthy controls that is not present in patients. Our current study design did not enable us to further investigate the possible synergetic effect of practice plus cerebellar cTBS, but it is an interesting topic for further study.

Acknowledging the small number of patients, no correlations between mean number of CRs and disease duration or severity score were observed, cautiously suggesting that abnormal EBCC is a trait characteristic of dystonia (also supported by altered cerebellar function in non-manifesting DYT1 gene carriers)[8]. It appears that the EBCC deficits and the underlying cerebellar abnormality seen in patients with CD might resemble those observed in patients with essential tremor (ET). Patients with ET show conditioning deficits that do not correlate with the degree of tremor or ataxia. Patients with ET also show normal timing of the CRs and the ability to improve the number of CRs in a subsequent conditioning block, and EBCC in them is normalized by thalamic deep brain stimulation [24, 25].

There are a few points that need to be addressed. The indirect effects of cerebellar cTBS are likely not limited to the cerebellar cortex, as recent positron emission tomography studies have shown that apart from direct reduction of cerebellar metabolism by inhibitory rTMS, secondary changes are seen in several cerebral areas [26, 27]. However, given that the cerebellum is the primary site for EBCC and that decerebrated animals are capable of EBCC, we consider it less likely that the positive effects of cerebellar cTBS on EBCC outcome result from more remotely modified areas [28].

When visualizing the time effect of cerebellar cTBS on EBCC in patients and controls (Figure 5), the effect of cerebellar cTBS seems larger in the first three blocks of EBCC in patients, whereas in healthy controls the effect over time was steady. This reduction over time could possibly be caused by (re-occurring) overactivation of the cerebellum in patients with dystonia, or might be explained by possible functional anatomical differences between patients and controls.

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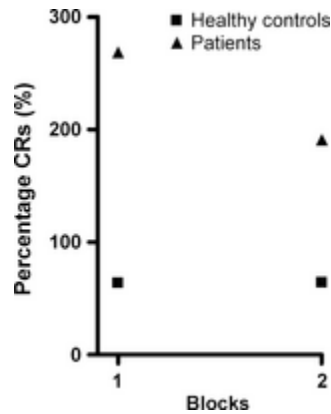
Figure 5 | In this figure, we visualized the effect of cerebellar cTBS over time in healthy controls and patients. *y*-axis: mean percentage of increase or decrease of conditioned responses (CRs). *x*-axis: 1 – mean of first three conditioning blocks studied; and 2 – mean of second three conditioning blocks studied.

We did not find any effect of cerebellar cTBS on motor cortical excitability in patients with CD. This contrasts a study by Koch et al. (2008) who reported that cerebellar cTBS decreases motor cortical excitability [29]. However, no significant differences in motor cortex excitability were observed after cerebellar cTBS in a recent study by Popa et al. (2010) or in our previous EBCC study [20, 30]. Methodological differences between our experiments and those of Koch et al. (2008), such as estimation of AMT, could account for these disparate findings on the effect of cerebellar cTBS on motor cortical excitability [29].

Abnormal blink reflex excitability has been demonstrated in primary dystonia, suggesting abnormal brainstem plasticity. Due to the time constraints of cerebellar cTBS effectiveness, we were unfortunately unable to study a possible alteration of blink reflex recovery following cerebellar cTBS. However, as our previous study of EBCC in dystonia indicated that EBCC deficits do not seem to originate from blink reflex abnormalities (Teo et al., 2009) and as the characteristics of the eyeblinks induced by supra-orbital nerve stimulation were not affected by cerebellar cTBS in this study or our previous study (Hoffland et al., 2012), we think that it is unlikely that our results of EBCC improvement are attributable to normalization of plasticity in the circuitry serving the blink reflex [15, 20].

Regarding the methods of our study, we did not include a sham group because this is a form of implicit learning that is not subjective to placebo-like effects. In our previous study, we included a sham group in which we applied cTBS over the neck muscles and ruled out the possibility that observed effects were due to (sensory) stimulation of non-cerebellar structures. Retention effects made it difficult to study the effect of cTBS on EBCC in the same patient group as we would then need to use an interval of several months perhaps to be certain of total extinction.

This is the first study to show that cerebellar abnormalities in dystonia are not static, but that a functional and partially reversible deficit of the olivo-cerebellar pathway appears to

be responsible for the EBCC deficits in primary dystonia. Cerebellar dysfunction could either be caused by compensatory engagement of the cerebellum, or by pathological recruitment of the cerebellum in the abnormal sensorimotor network in dystonia and as such in part responsible for the clinical expression of dystonia [31]. This issue is not settled by the data presented here, and we encourage future studies in which the effects of cerebellar cTBS and/ or inhibitory theta burst stimulation on the actual dystonic symptoms are investigated.

Chapter 4.2

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Chapter 4.2

4.3

Normal eyeblink classical conditioning in patients with fixed dystonia.

Abstract

Fixed dystonia without evidence of basal ganglia lesions or neurodegeneration typically affects young women following minor peripheral trauma. We use eyeblink classical conditioning (EBCC) to study whether cerebellar functioning is abnormal in patients with fixed dystonia, since this is part of the pathophysiology of primary dystonia. An auditory tone (conditioning stimulus) was paired with a supraorbital nerve stimulus (unconditioned stimulus) with a delay of 400 ms in order to yield conditioned responses. We recruited 11 fixed dystonia patients of whom six used medication and seven age-matched healthy controls. Non-medicated patients with fixed dystonia performed as well as healthy controls, while medicated patients showed fewer conditioned responses. We found an influence of medication and possibly extent of dystonic features and/or co-occurrence of complex regional pain syndrome (CRPS) on EBCC performance. Our study argues against abnormal cerebellar function in non-medicated, fixed dystonia patients without CRPS or spread of symptoms.

Introduction

Dystonia is characterized by abnormal co-contraction of agonist and antagonist muscle groups causing twisting movements and abnormal postures of the affected body parts[1]. Fixed, immobile dystonic postures can occur in secondary dystonia due to, amongst others, acquired basal ganglia lesions, corticobasal degeneration or cerebellar lesions [2, 3]. However, in a considerable group of patients who develop fixed dystonic postures, no cause can be found. In these patients, isolated fixed dystonic postures typically develop in young women, in a majority of cases starting in a limb following peripheral tissue injury with spontaneous spread to other body regions [3]. More than one-third of these patients have complex regional pain syndrome type 1 (CRPS1) [4].

Fixed dystonia is at present mainly regarded to be a functional disorder and considered within a biopsychosocial model [5]. In primary dystonia, there is accumulating evidence for a pathophysiologic role for the cerebellum [2]. Cerebellar functioning can be assessed by using eyeblink classical conditioning (EBCC), a neurophysiologic paradigm which is predominantly dependent upon the inferior olive and cerebellum [6, 7]. In EBCC, associative motor learning is tested by repeatedly pairing a conditioning stimulus (CS) with an unconditioned stimulus (US), which leads to the production of conditioned responses (CR). The main neurocircuitries involved in mediating the US and CS include the olivo-cerebellar and ponto-cerebellar systems, respectively [8]. Teo et al. (2009) and Hoffland et al. (2013) have demonstrated lower acquisition of CRs and impaired extinction in patients with adult-onset focal dystonia, indicating functional changes in the olivo-cerebellar and ponto-cerebellar pathway in patients with primary dystonia[9, 10]. We here investigate whether cerebellar function is also altered in patients with fixed dystonia using the EBCC paradigm.

Methods

Subjects

Table 1 summarizes patient characteristics. Eleven patients with fixed dystonia were included (all women, mean age 43.6 ± 15.8 years; range 18–65 years). Six patients used medication, four patients also fulfilled the IASP diagnostic criteria for CRPS (Merskey et al. 1994). Dystonia remained limited to the site of onset in four patients, had spread to adjacent body parts in six patients and was generalized in one patient. The mean disease duration was 9.5 ± 6.8 years with a mean onset at the age of 32.1 ± 9.1 years. Seven age-matched healthy controls (six women, mean age 49.7 ± 15.6 years; range 25–65 years) who did not use medication and had no history of neurological, psychiatric or hearing disorders were included. All participants had given informed consent prior to inclusion. The study was approved by the local ethics committee of the Radboud University Nijmegen and was conducted according to the standards set by the Declaration of Helsinki.

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Table 1 | Patient characteristics

P#	Group	Sex	Age	Site of onset	Precipitating factor	Spread	Age of onset (years)	Disease duration (years)	Medication (daily)
P1	NM	F	50	Right shoulder	Unknown	Generalized	35	17	–
P2	NM	F	18	Left hand (digit 3, 4 and 5)	Cleaning dishes	Stable to site of onset	16	P2	NM
P3	NM	F	44	Right foot	Foot injury treated with plaster	Stable to site of onset	36	8	–
P4	NM	F	34	Both feet	Unknown	Stable to site of onset	32	2	–
P5	NM	F	29	Left leg	Hip replacement operation	Stable to site of onset	24	5	–
P6	M	F	50	Left leg	Physical therapy neck	Spread to left arm, hand, torso, right leg and arm (generalization)	44	6	Fentanyl transdermal 100 µg/h every 48 h; atenolol, 50 mg
P7	M	F	22	Left leg	Distorsion ankle; CRPS (since age 15)	Spread to right leg	18	4	Clonazepam, 500 µg; baclofen, 80 µg; tramadol, 150 mg; amitriptyline, 20 mg
P8	M	F	50	Both feet	Surgery dig I both legs; CRPS	Gradual progression in both feet	33	17	Baclofen, 75 µg; oxycodon, 120 mg
P9	M	F	57	Right hand	Wrist fracture; cast; CRPS	Spread to left leg	38	19	Buprenorphine, 0.8 mg; domperidone, 40 mg; meloxicam 15 mg/day
P10	M	F	60	Right foot	Surgery rupture fascia lata; CRPS	Spread to both hands	42	18	Temazepam, 10 mg; amitriptyline, 25 mg; magnesium oxide 2.5 g
P11	M	F	41	Both legs	Surgery benign tumour breast	Spread to both arms	35	6	Clonazepam, 500 µg; carbamazepine 600 mg; botulinetoxine A, last dose 4 weeks earlier

M medicated, NM non-medicated

Eyeblink conditioning

A loud auditory tone of ~2 kHz and 400 ms played via binaural headphones was used as CS. After an interval of 400 ms, the CS was followed by the US: a single electrical stimulus over the right supraorbital nerve with a pulse width of 200 μs and an intensity of 7–10 times the sensory threshold delivered through an electrical stimulator Digitimer DS7A (Digitimer Ltd). Surface electromyographic (EMG) activity was recorded using Ag–AgCl electrodes from the right orbicularis oculi muscle.

The conditioning paradigm consisted of six acquisition blocks of 11 trials each. In trials 1–9, the CS and US were paired, trial 10 was CS-only in order to verify that CRs were being acquired independent from the US, and trial 11 was US-only to monitor if the stimulus remained effective in producing unconditioned responses (URs). The six acquisition blocks were followed by an extinction block consisting of 11 CS-only trials to measure extinction. The inter-trial interval was randomized between 10 and 30 s to reduce habituation.

CRs were defined as EMG activity of at least five times the standard deviation above the mean, as calculated from the EMG baseline activity, with a clear rising slope, occurring within 250 ms prior to the US and lasting at least 50 ms or merging into a superimposed UR in paired trials and within 150–600 ms after the CS in CS-only trials. EMG bursts were regarded acoustic startle responses ('α-blinks') if their amplitude exceeded 50 μV and if they occurred within 150 ms after the CS. The time to onset, time to maximum ('peak latency') and the maximum of the rectified EMG amplitude ('magnitude') of CRs and URs were automatically quantified using custom written software in Matlab 7.6.0 R2008A (The MathWorks, Natick, MA) and were checked visually.

Data analysis and statistics

To determine the influence of medication on EBCC performance, three groups were defined: 'medicated patients', 'non-medicated patients' and 'healthy controls'. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL) version 18.0. The mean percentage CRs over the six acquisition blocks, mean percentage CRs of the extinction blocks, mean magnitude, mean onset and peak latency of the CRs, mean peak latency and magnitude of the URs, mean percentage of α-blinks, age, gender and stimulus intensity were used as dependent variables. The Shapiro–Wilk test of normality showed that the data were not normally distributed and therefore a Kruskall–Wallis test was used to analyse group effects (reported as χ^2 values). Mann–Whitney U tests were carried out as post hoc tests (reported as Z approximations). A Wilcoxon signed-rank test was used to test the percentage of CRs between the sixth block and extinction block.

Results

All participants completed the experiment. The median electrical stimuli intensity used was 15.6 (range 15.6–35.0) mA in non-medicated patients, 34.0 (range 3.5–50.0) mA in medicated patients and 36.0 (range 30.0–70.0) mA in healthy controls. There were no significant group

effects in the three groups for the mean age ($\chi^2 = 3.745$, p = 0.154), gender ($\chi^2 = 1.571$, p = 0.456) or electrical stimulus intensity ($\chi^2 = 5.241$, p = 0.073). Disease duration did not differ between the two patient groups (Z = −1.372, p = 0.170).

Acquisition of conditioned responses

The percentages of CRs per block are visualized in Figure 1. There were significant group effects between controls, medicated patients and non-medicated patients for the mean of the first six blocks ($\chi^2 = 6.432$, p = 0.040).
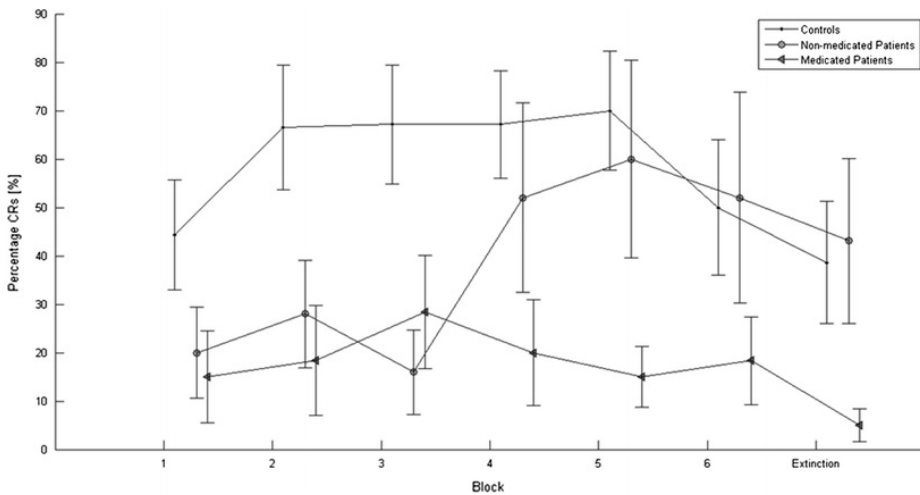
Figure 1 | The percentages of CRs in each block for the three groups ('controls', 'non-medicated patients' and 'medicated patients'). The bars indicate standard error.

When compared to the control group, medicated patients had a significantly lower mean percentage CRs in the six acquisition blocks (Z=-2.375, p=0.018), while non-medicated patients did not (Z=-1.218, p=0.223).

The percentage of CRs did not significantly differ between the sixth block and the extinction block (Z = −1.882), p = 0.060. There were no significant differences in the mean onset and peak latency, and mean magnitude of CRs.

α-blinks

There was no significant group effect ($\chi^2 = 5.753$, p = 0.056) for the mean of the six acquisition blocks.

Unconditioned responses

No significant differences were found when comparing mean magnitudes or mean peak latencies of the URs during the acquisition blocks.

Discussion

This study used EBCC to investigate whether cerebellar functioning is altered in patients with fixed dystonia. When non-medicated fixed dystonia patients were tested against healthy controls, no significant differences in mean percentage of CRs or other parameters were found. This contrasts the study by Teo et al. (2009) that showed cerebellar dysfunction in patients with primary focal dystonia, suggesting different pathophysiological mechanisms in fixed and primary dystonia[10]. This is in line with a study by Quartarone et al. (2009), which demonstrated that cortical plasticity was abnormal in organic dystonia, but normal in fixed dystonia[11]. Conversely, other studies have demonstrated abnormalities in cortical inhibition in both fixed and primary dystonia, but the measures used were nonspecific and do not indisputably prove a shared biological mechanism[12, 13].

Patients using medication developed significantly fewer CRs during the acquisition blocks when compared to healthy controls. This suggests an overall effect of medication on olivo-cerebellar and ponto-cerebellar pathways influencing EBCC performance, which has already been suggested by studies investigating cerebellar involvement in the pathogenesis of schizophrenia [14]. However, it should be noted that in four non-medicated patients, dystonic features remained limited to the site of onset and none had CRPS, while in all medicated patients symptoms spread to other body parts and four had CRPS. It is possible that the co-occurrence of CRPS and/or extent of dystonic features correlate with EBCC performance, rather than or in addition to an effect of medication. Another notable point is that the pattern of CRs over the six blocks in non-medicated patients was characterized by a slow start, although a sharp increase from block four resulted in an equal ending of the percentage of CRs compared to controls. We can therefore not exclude that a slower learning rate is present in non-medicated, fixed dystonia patients. Another explanation for the relatively slower start could be the lower electrical stimulus in this group. However, lower stimulus intensities are expected to reduce EBC performance, and considering the fact that non-medicated patients acquired as many CRs as controls despite the lower intensities used, it is unlikely that this has affected our main findings. The previously mentioned studies by Teo et al. (2009) and Hoffland et al. (2013) showed that patients with adult-onset focal dystonia had a lower increase in CRs during the acquisition blocks and accordingly had a lower mean number of CRs compared with healthy controls[9, 10]. In the current study, non-medicated patients and healthy controls (eventually) acquired equal rates of CRs. Therefore, we consider it unlikely that major impairments in EBCC performance, such as those that exist in patients with adult-onset focal dystonia, exist in non-medicated, fixed dystonia patients.

The number of CRs during the extinction block was not significantly different between the last acquisition block (block 6) and the extinction block (block 7), which does not support clear extinction in this phase. However, the p value of 0.06 suggests a trend towards significance. Possibly, extinction would have been statistically significant if tested in more individuals, or if a higher number of CRs was acquired at the start of the extinction block, e.g. by increasing the number of acquisition blocks.

A limitation of our study is the relatively small number of subjects, and the size of subgroups was smaller than our group of organic dystonia patients in whom we showed abnormal EBCC previously [10]. This might have led to our study being underpowered to detect smaller differences in EBCC performance.

In summary, our findings argue against abnormal cerebellar function in non-medicated fixed dystonia patients without CRPS or spread of symptoms, pointing to a different neurobiological background in fixed versus primary dystonia. However, given our small patient population and since we only investigated neurophysiological evidence of cerebellar dysfunction, we encourage further studies exploring the subject.

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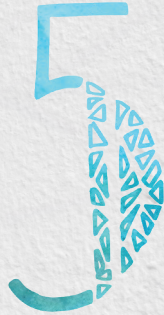
Chapter 4.3

5

A Gait Paradigm Reveals Different Patterns of Abnormal Cerebellar Motor Learning in Primary Focal Dystonias.

Abstract

Accumulating evidence points to a role of the cerebellum in the pathophysiology of primary dystonia. The aim of this study was to investigate whether the abnormalities of cerebellar motor learning in primary dystonia are solely detectable in more pure forms of cerebellum-dependent associative motor learning paradigms, or whether these are also present in other motor learning paradigms that rely heavily on the cerebellum but in addition require a more widespread sensorimotor network. Twenty-six patients with various forms of focal dystonia and 10 age-matched healthy controls participated in a motor learning paradigm on a split-belt treadmill. By using reflective markers, three-dimensional kinematics were recorded using a 6-camera motion analysis system. Adaptation walking parameters were analyzed offline, comparing the different dystonia groups and healthy controls.

Patients with blepharospasm and writer's cramp were significantly impaired on various adaptation walking parameters. Whereas results of cervical dystonia patients did not differ from healthy controls in terms of adaptation walking parameters, differences in parameters of normal gait were found. We have here demonstrated abnormal sensorimotor adaptation with the split-belt paradigm in patients with blepharospasm and writer's cramp. This reinforces the current concept of cerebellar dysfunction in primary dystonia, and that this extends beyond more pure forms of cerebellumdependent associative motor learning paradigms. However, the finding of normal adaptation in cervical dystonia patients indicates that the pattern of cerebellar dysfunction may be slightly different for the various forms of primary focal dystonia, suggesting that actual cerebellar pathology may not be a primary driving force in dystonia.

Introduction

Dystonia is a movement disorder characterized by involuntary muscle contractions resulting in abnormal postures and twisting movements. The basal ganglia are traditionally considered to be key player in the pathophysiology of dystonia, but accumulating evidence currently points to an additional role of the cerebellum. For example, eyeblink classical conditioning (EBCC), a cerebellum-dependent paradigm of associative motor learning, was found to be abnormal in primary focal dystonia [1]. Cerebellar dysfunction in dystonia can firstly be due to pathological involvement of the cerebellum, but secondly also to a more compensatory engagement. The theory here then would be that the compensatory recruitment of the cerebellum is detrimental for other cerebellar tasks, such as associative motor learning. Interestingly, we recently showed that the EBCC deficits in primary dystonia can be modified by practice and also by direct noninvasive modulation of cerebellar excitability (through inhibitory continuous theta burst stimulation) [2]. This suggests that these functional deficits are reversible, which argues against a structural cerebellar defect.

The aim of this study was to investigate whether the abnormalities of cerebellar motor learning in primary dystonia are solely detectable in more pure forms of cerebellum dependent associative motor learning paradigms, such as EBCC, or whether these are also present in other motor learning paradigms that rely heavily on the cerebellum but in addition require more a widespread cerebral sensorimotor network. For this, we chose a motor learning paradigm on a split-belt treadmill [3]. Two types of gait adjustments are seen during split-belt walking: (1) direct reactive adjustments of walking parameters (e.g., stride length and time instance) to accommodate the novel difference in belt speeds and (2) adaptive feed forward adjustments in step length, time in double support, oscillation, and phasing parameters. Both cats with a transection of the spinal cord [4] and human infants [5] can make reactive feedback adaptations during split-belt treadmill locomotion, and these adjustments therefore are thought to predominantly or exclusively rely on spinal circuitry.

Feed forward sensorimotor adaptation is, however, a more gradual form of motor learning used to adjust movements over minutes to hours in response to a certain perturbation, which leads to storage of a new motor pattern, which also persist as aftereffects when the perturbation is removed. The cerebellum is thought to be essential for this feed forward component as this paradigm challenges the ability to modify motor programs for adapting posture and locomotion through trial-and-error practice, which relies on the cerebellum [3]. We therefore expected to mainly pick up abnormalities in these adaptive feed forward adjustments if there are indeed more extensive cerebellar learning difficulties in dystonia patients. Furthermore, the treadmill set-up also allowed us to assess normal gait and tandem gait walking to investigate possible subtle (cerebellar) gait abnormalities in dystonia patients. Prior studies have shown that in other movement disorders in which the clinical impression is that gait is normal, such as essential tremor, subclinical gait abnormalities can still be detected [6, 7].

Methods
Subjects
Twenty-six dystonia patients (mean age 56.5±8.2; 13 males) and 10 age-matched healthy controls (mean age 54.8±7.8 years; 4 males) participated in the study after giving their written informed consent. The patient group consisted of three subgroups: 10 cervical dystonia (CD) patients (mean age 54.0±7.4; 4 males), 9 blepharospasm (BSP) patients (mean age 60.8±8.6; 4 males), and 7 writer's cramp (WC) patients (55.3±8.8; 5 males). The study was approved by the local ethics committee. Patients who received botulinum toxin treatment were investigated in between treatments, e.g., when the effect of their last botulinum toxin injection was maximal. All patients demonstrated very good resolution of symptoms at this time. This was done to minimalize possible negative effects of head movement or involuntary blinking on task execution in patients.

Recording
Twenty-six reflective markers were placed on anatomical landmarks according to the Vicon Plug-in- Gaitmodel. Three-dimensional kinematics were recorded by a 6-camera motion analysis system (Vicon Motion Systems, Oxford, UK) at a sample rate of 100 Hz.

Paradigm
A split-belt treadmill was used, consisting of two separate belts with independently controllable speeds (ForceLink BV, The Netherlands) [5, 8]. Subjects walked for 2 min at a speed of 3.6 km/h to investigate normal walking. After a short break, subjects were instructed to walk tandem (place one foot in front of the other, touching toe to heel) at 1.0 km/h on a 3-cm wide line that was projected on the treadmill. Two 1-min trials were collected. Preferred walking speed was determined for each subject by two separate measurements on the treadmill. In addition, subjects filled out the Dutch version of the activities-specific balance confidence scale (ABC-NL) [9]. These two measurements were collected to assess normal gait in dystonia patients. The split-belt experimental procedure consisted of different testing periods with the two belts either moving at same (tied belt) or different speeds (split-belt). In tied belt configuration, both belts were set to move at slow (1.3 km/h) or fast speed (3.9 km/h). During split-belt walking, the right belt was set at high speed (3.9 km/h) and the left belt was set at slow speed (1.3 km/h). We wanted to use a 3:1 ratio for split-belt walking as the largest adaptation and aftereffect are seen when the speed ratio between the two legs is greatest [10]. Previously, studies using the split-belt paradigm chose 0.5 and 1.5 m/s for this 3:1 ratio. Given that some of our patients would not be able to sustain walking at these different speeds, we used a 1.3–3.9 km/h (0.36– 1.08 m/s) ratio. The paradigm consisted of a baseline period of 2 min walking at slow speed (S1), then subjects walked 2 min at fast speed (F), and again 2 min at slow speed (S2), all in the tied belts configuration. In the subsequent adaptation period (A), belts were switched to split-belt condition for 10 min. For the post-adaptation (P) period, subjects walked for 10 min at the slow tied belt configuration (Figure

1). Subjects were instructed not to look down at the belts while walking; during treadmill walking, one of the investigators stood at the front of the treadmill for safety reasons and to ensure participants did not look down during the experiment. The participants knew when the investigator changed the speed of the treadmill; however, they were not aware of the details of this change during the split-belt experiment. Variable leg dominance, differences in preferred walking speed, and differences in side of dystonia symptoms could possibly influence adaptation, but as this was a first explorative study, we chose to use an identical setup for all groups of participants.

L slow	L fast	L slow	L slow	L slow
R slow	R fast	R slow	R fast	R slow
S1	F	S2	A	PA

Figure 1 | Split-belt experimental set up: S1 (slowwalking 1) tied belt period of 2min walking at 1.3 km/h. F (fast walking) tied belt period of 2 min walking at 3.9 km/h. S2 (slow walking 2) tied belt period of 2 min walking at 1.3 km/h. A (adaptation) split-belt period (right belt 3.9 km/h and left belt 1.3 km/h) of 10 min. PA (post-adaptation) tied belt period of 10 min walking at 1.3 km/h.

Table 1 | Participant characteristics.

	Controls	All patients	WC	BSP	CD
Mean ± SD					
Number of subjects	10	26	7	9	10
Male [%]	40	50	71	44	40
Age [years]	54.8±7.77	56.5±8.2	55.3±8.8	60.8±8.6	54.0±7.4
Pref. walking speed [km/h]	4.6±0.5	3.9±0.9	4.6±0.8	3.4±0.6	3.7±0.9
Balance scale [%]	99.0±0.9	90.9±9.5	97.8±2.1	84.0±11.0	93.0±6.

Data analysis

Marker position data were analyzed offline using custom written software inMATLAB (The MathWorks, Natick, USA). The four main walking parameters used in the analysis of split-belt adaptation are the following: step length symmetry, double support (DS) time symmetry, oscillation, and phasing [8, 11]. The leg that was adapted on the slow belt will be referred to as the 'slow leg' and the leg on the fast belt as the 'fast leg' (even during tied belt walking). We also calculated the stance to swing time ratio, step width, foot angle, and an ataxia ratio to investigate possible subtle gait differences during normal walking and tandem gait. For the tandem gait recordings, the numbers of missteps (steps not hitting the projected line) were

counted as well. Given that this number turned out to be very low, it was discarded from further analysis. For more information regarding the definitions and calculations of the above parameters, we refer to the supplementary methods. To investigate the overall course of the split-belt experiment, we investigated walking over five different time periods. We therefore calculated the mean of the first five steps for slow walking, adaptation, and post-adaptation (S2, A-beginning, and P-beginning) and the mean of the last five steps of adaptation and post adaptation (A-end and P-end). Baseline values (mean of first five of last 30 s S2) were subtracted to normalize this data. To investigate if significant adaptation of these variables had occurred during split-belt walking, we first investigated whether P-beginning was significantly different from null in the different groups. Feed forward adjustments require practice to obtain and result in storage of a new movement pattern, observed as negative aftereffects after returning to the original condition. So, if there is no significant difference between baseline and post adaptation in a selected variable, no significant aftereffect is observed in this group. This indicates aberrant adaptation. To compare the time course and development of step length adaptation, a rate of adaptation was calculated for step length symmetry. Walking parameters were averaged in bins of 10 steps for the first 210 steps of adaptation for each subject; corresponding to duration of 279.2±45.6 s on average, i.e., about half the adaptation period. To fairly compare this rate of adaptation, data was rescaled by their respective starting point, thus expressed as a percentage of the mean values over the first five steps of adaptation. Data of one BSP patient was discarded from analysis of the split-belt experiment because of technical malfunctioning. Data of two CD patients was not available for normal walking analysis and data of one subject with CD for tandem gait analysis because of technical malfunctioning.

Statistics

Patient characteristics were tested with an ANOVA over GROUPs.Walking parameters during tandemgait and normal walking were tested using a one-way ANOVA with GROUP as between-subject factor (controls, WC, BSP, and CD). Adaptation aftereffects were first investigated with a one sample T-test in which P-beginning was tested against null to see if significant adaptation occurred. After this, a repeated measures ANOVA was performed on the five different walking periods with between-subject factor GROUP (controls, WC, BSP, CD) and within-subject factor PERIOD (S2, A-beginning, A-end, P-beginning, P-end). Finally, for the adaptation rate, we tested the number of steps (in bins of 10 steps) participants took to adapt to 50% of their starting point using a one-way ANOVA with between subject factor GROUP. If 50 % adaptation was not reached after 210 steps, this maximum value was used in the analysis. In the case of a significant main effect, post hoc comparisons were made using LSD tests. Pearson's correlation coefficients were calculated for all walking variables versus the ABC-NL outcomes. Significance was set at p less than 0.05, unless stated otherwise.

Results

Participant characteristics

There were no GROUP differences in age between controls and the patient groups ($F_{(3)}$= 1.194, p=0.327). The same applies to the proportion of male subjects ($χ2_{(3)}$=2.09, p=0.55) (Table 1).

Preferred walking speed

For the preferred walking speed, there was a GROUP effect ($F_{(3)}$=6.860, p=0.001). The preferred walking speed in CD and BSP patients was lower compared with controls (p=0.005 and p=0.001, respectively) and compared with patients with WC (p=0.013 and p=0.003, respectively) (Table 1).

Balance confidence

Also the ABC-NL yielded significant GROUP effects ($F_{(3)}$=9.791, p<0.001). Post-hoc tests revealed reduced confidence in one's own balance in BSP versus all other groups (p≤0.005) and in CD versus controls (p=0.046) (Table 1).

Normal walking

No significant differences were found for step width, swing/stance ratio, or step length symmetry. However, for the normal walking measures, there was a significant difference between GROUPS for the ataxia ratio (p=0.035, $F_{(3)}$=3.251). According to post hoc tests, patients with BSP had a more variable gait than those with WC (p=0.004).

Tandem gait

No GROUP effects were found for any of the tandem gait walking measures (step width, swing/stance ratio, foot angle, ataxia ratio, or step length symmetry).

Aftereffect

To investigate if adaptation had occurred, the presence of an aftereffect was investigated by testing P-beginning against null using a one sample T-test for different groups. In healthy controls, P-beginning significantly differed from zero for all variables (step length $t_{(9)}$=4.51, p=0.001, double support time $t_{(9)}$=3.46, p=0.007, oscillation $t_{(9)}$=−2.92, p=0.017, and phasing $t_{(9)}$=−6.55, p<0.001). Also in CD patients, P-beginning significantly differed from zero for all variables (step length $t_{(9)}$=6.14, p<0.001, double support time $t_{(9)}$=6.14, p<0.001, oscillation $t_{(9)}$=−3.09, p=0.013, phasing $t_{(9)}$=3.28, p=0.010). In BSP patients, P-beginning did not significantly differ from zero for step length ($t_{(7)}$= 2.35, p=0.051), for double support time ($t_{(7)}$=1.15, p=0.29), and for oscillation ($t_{(7)}$=−1.92, p=0.097). In BSP patients, P-beginning significantly differed from zero for phasing ($t_{(7)}$= −3.30, p=0.013). In WC patients, P-beginning did not significantly differ from zero for double support time ($t_{(6)}$= −0.48, p=0.65) or for

Chapter 5
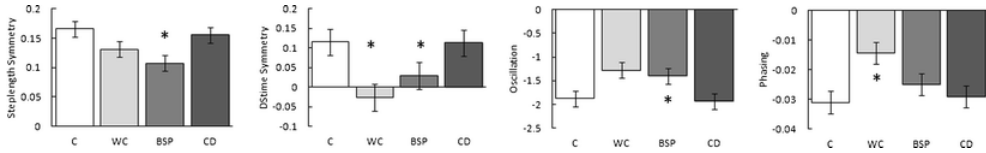
Figure 2 | Aftereffect. Asterisk indicates P-beginning did not significantly differ from null.

phasing (t(6)=−1.24, p=0.27). In WC patients, P-beginning significantly differed from zero for step length (t(6)=6.35, p=0.001) and oscillation (t(6)=−2.68, p=0.037) (Figure 2).

Walking parameters for the five periods

Running a rANOVA over the five different periods did not result in significant group or PERIOD*GROUP interactions for steplength symmetry, oscillation, or phasing. For DS symmetry, both PERIOD*GROUP (F(3)=3.547, p=0.026) and GROUP (F(3)=5.157, p=0.005) were significant. When testing the periods separately, there was only a significant GROUP effect for DS time symmetry on A-end, P-beginning, and P-end (F(3)=3.994, p=0.016; F(3)=3.242, p=0.035; F(3)=4.902, p=0.007). For A-end, significant differences existed between WC and all three other groups (p<0.02); for P-beginning, between WC and controls (p=0.013) and CD (p=0.016); and for P-end between controls and all other groups (p<0.025) (Figure 3).
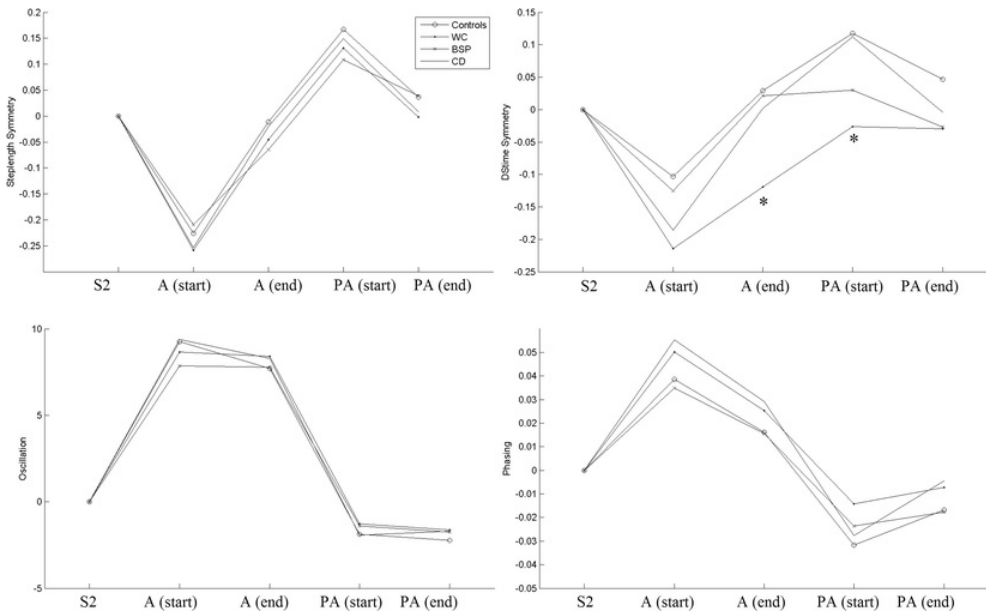
Figure 3 | Walking parameters (step length symmetry, double support time symmetry, oscillation, phasing) over the five periods with baseline (S2) subtraction, visualized per group (controls, WC, BSP, CD).

Adaptation rate

There was no difference in the mean first five steps of step length symmetry between all groups (F(3)=0.167, p=0.918). There was a significant GROUP effect for the number of steps it took for participants to adapt to 50 % of their starting point (F(3)=4.311, p=0.012). LSD post hoc tests showed differences between controls and WC (p=0.018) and BSP (p=0.017), between BSP and CD (p=0.014) and between WC and CD (p=0.016). Adaptation was thus significantly slower in patients with BSP and WC compared to the healthy controls and to CD patients (Figure 4).
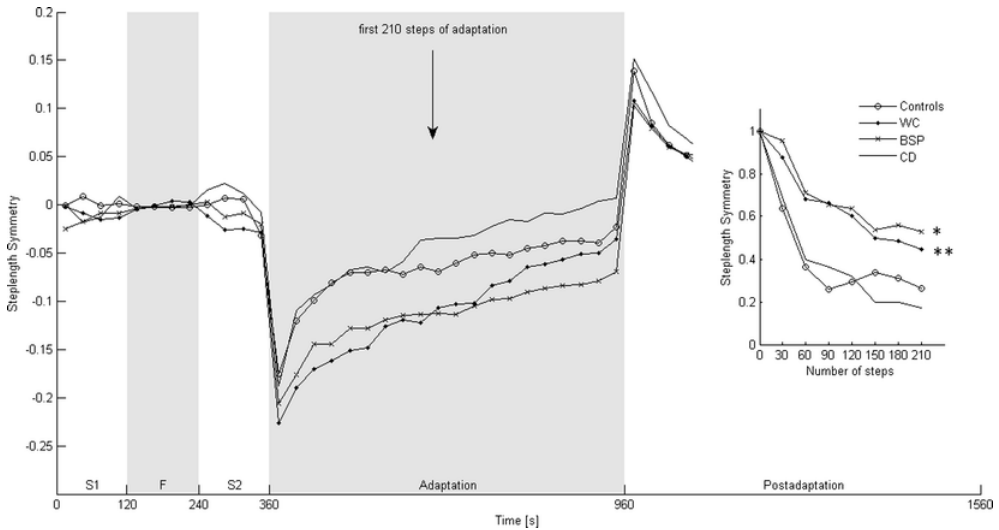
Figure 4 | Larger picture: Raw data of mean step length symmetry per 30 s for all four groups (controls, WC, BSP, CD) during the whole walking paradigm: slow walking S1 (2 min), fast walking F (2 min), slow walking S2 (2 min), adaptation (10 min) and post adaptation (10 min). The arrow indicates the first 210 steps of adaptation as used in analysis of the adaptation rate. Smaller inset: Step length symmetry averaged in 7 bins of 30 steps; rescaled by their respective starting point.

Discussion

There are other studies that addressed cerebellar learning capacities in patients with dystonia, and the tasks used were eye-blink classical conditioning (EBCC; a form of cerebellum-dependent associative motor learning) and sequence learning. The observed EBCC deficits were shown to be modifiable [2] and the cerebellum was shown to be hyperactive during sequence learning [12]. These studies let us believe that cerebellar abnormalities in dystonia are functional and possibly even compensatory in nature. In line with this is the lack of overt clinical sings of cerebellar dysfunction, including gait, in patients with primary dystonia. However, sensorimotor adaptation has to our knowledge never been studied in primary dystonia. The cerebellum is thought to be essential for sensorimotor adaptation, and the paradigm we have used here challenges the ability to modify motor programs for adapting posture and locomotion through trial-and error practice, which relies on the cerebellum.

Chapter 5

We investigated feed forward sensorimotor adaptation with the split-belt paradigm [3] to study the cerebellar control of locomotion in various forms of primary, focal dystonia. We deliberately chose to study various types of focal dystonia to comment on the issue of convergence or divergence of pathophysiological mechanisms in these different subtypes. In addition, we studied normal gait and tandem gait to investigate possible subtle gait differences between patients and controls.

The main limitation of our study is the relatively low number of patients per dystonia subgroup. As movement data was automatically generated from marker position data using custom written software, bias from non-blinded evaluations is expected to be small. Our results need to be confirmed by others and we encourage further studies of cerebellar motor learning in primary dystonia.

Normal and tandem gait

This study of gait firstly shows that patients with blepharospasm (BSP) and cervical dystonia (CD) prefer a lower walking speed and have significantly lower balance confidence compared to healthy controls (C) and patients with writer's cramp (WC). During normal walking, BSP patients differed significantly from WC on the ataxia ratio, reflecting less constant execution of movement in BSP that might result from cautious walking. Therefore, subtle kinematic gait abnormalities might exist in some forms of primary focal dystonia, but none of the gait abnormalities we observed were considered sufficiently indicative of cerebellar dysfunction, as in particular there were no tandem gait difficulties. Other explanations in the BSP group could be reduced visual input, or that—as mean age of BSP patients was slightly higher than the other three groups—there is more of an age-related reduction in walking speed [13]. For CD, the explanation for a reduced balance confidence might lie in the abnormal head posture during walking, or in abnormal vestibular input as a consequence of this [14].

Sensorimotor adaptation

Multiple abnormalities where observed in BSP and WC patients during split-belt walking. Feed forward sensorimotor adaptation is used to adjust movements over minutes to hours in response to a certain perturbation, which leads to storage of a new motor pattern and therefore to aftereffects when the perturbation is removed. When testing the beginning of post-adaptation against zero to see if significant aftereffects (and therefore adaptation) had occurred, P-beginning did not significantly differ from zero for step length, double support time, or oscillation in BSP patients. In WC patients, P-beginning did not significantly differ from zero for double support time and phasing. When testing split-belt walking during the five different periods of the experiment, significant differences were observed for DS time values with WC patients showing abnormal adaptation. The rate of adaptation of step length symmetry was significantly different between patient groups, as WC and BSP patients displayed a slower rate of adaptation compared to patients with CD and healthy controls. The speed of step length symmetry adaptation can be influenced by changing both spatial

(oscillation) and temporal (DS time, phasing) parameters of walking. So, for WC patients, a significant slower rate of adaptation probably resulted from abnormalities in temporal adaptation (DS time and phasing), whereas slower adaptation in BSP patients seems to be attributable to abnormalities in both spatial (oscillation) and temporal adaptation (double support time). The presently observed deficits in adaptations may be further indicators of cerebellar dysfunction in dystonia. Patients with cerebellar degeneration show impairments in both the spatial and temporal domains of splitbelt adaptation [3]. However, differential modulation of spatial and temporal parameters by conscious correction or by cerebellar transcranial direct current stimulation [8, 15] suggests that there are different neural substrates for the spatial versus temporal components. It has been hypothesized that the pontocerebellum (which projects to cortical regions) could play a stronger role in the spatial control of locomotion and that the spinocerebellum (projecting to the vestibulospinal and reticulospinal tracts) might be more involved in temporal coordination. Interestingly, patients with WC and BSP showed different abnormalities in these adaptation parameters, whereas patients with CD were not significantly impaired on this task. This might suggests different patterns, in terms of degree and area, of cerebellar malfunctioning in these different forms of primary dystonia.

We have recently demonstrated abnormal EBCC in CD patients [2] but found no clear abnormalities in this split-belt motor learning paradigm. This could be due to the fact that cerebellar dysfunction in CD is not as widespread as in other forms of focal dystonia. Of interest, Schwingenshuh et al. also observed similar levels of motor adaptation in CD patients and controls using a different task, although they did observe that patients might use a different strategy to complete a motor sequence learning task. They did not test other forms of dystonia [16].

In summary, we have demonstrated abnormal sensorimotor adaptation with the split-belt paradigm in patients with blepharospasm and writer's cramp. This reinforces the current concept of cerebellar dysfunction in primary dystonia and indicates that this abnormality extends beyond more pure forms of cerebellum-dependent associative motor learning paradigms. However, the finding of normal adaptation in cervical dystonia patients indicates that the pattern of cerebellar dysfunction is slightly different for the various forms of primary focal dystonia, but also argues against actual cerebellar pathology as a primary driving force in dystonia.

Chapter 5

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Supplementary material
Split-belt treadmill

Subjects were positioned in the middle of the treadmill, with one foot on each belt. All subjects wore a safety harness and held onto a rail in front of them at elbow height during the split-belt walking paradigm. They were instructed not to look down at the belts while walking (except when studying tandem gait). The experiment started with a practice period to make subjects familiar with walking on a treadmill.

Walking parameters

Double support (DS) time was defined as the time during which both feet were in contact with the ground. The percentage DS time was calculated as the time from initial ground contact of one limb to lift-off of the other limb, expressed as a percentage of total stride time (the time between two consecutive heel contacts of the same foot) of the lift-off limb. These measures were calculated for both limbs. To measure inter-limb coordination, symmetry parameters for step length and DS time were calculated. These symmetry parameters were defined as the difference between fast and slow leg divided by the sum of the slow and fast leg, as introduced by Vasudevan et al [5].

It has been found that step length can be altered by adapting spatial and temporal elements of coordination[3]. For the spatial component, step length is adapted by changing the angle about which the limbs oscillate. This oscillation was calculated for each step as the midpoint angle of the limb angle between heel strike and toe off for each leg. The limb angle refers to the angle between Vicon's calculated hip joint centre and toe marker with respect to the vertical axis. The temporal element is called limb angle phasing and was calculated as the time lag at peak of half the cross-correlation spectrum of both limb angles over one stride cycle. The slow leg was used as the reference lag here. Phasing values ranged from 0 to 1; a phase of 0.5 indicates perfect out-of-phase walking. Again, the difference between both legs was calculated.

The stance phase is the time between initial heel contact to toe off of the same limb and swing time is exactly the opposite period, in which the foot is not in contact with the ground. Step width was the distance between the right and left heel marker at ground contact of both feet per step. Foot angle was calculated as the average angle during stance between toe and heel with respect to the forward line of progression and averaged between limbs and used to analyse tandem gait walking. Step height was the difference between the maximum and minimum height of the heel marker per step and used to calculate the ataxia ratio. The ataxia ratio was defined as the mean standard deviation (SD) of foot placement in all three directions (SD of step width + SD of step height + SD of step length / 3) [6].

Cerebellar and basal ganglia contributions to motor learning in idiopathic focal dystonia.

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Abstract

The basal ganglia and cerebellum are though to play main roles in the pathophysiology of dystonia, but their nature of involvement (particularly for the cerebellum) and their interaction remains a matter of debate. The differential involvement of these two brain regions in various forms of motor learning offer an opportunity to further investigate this.

We used fMRI to evaluate cerebellar and basal ganglia alterations in functioning during visuomotor learning (a motor task thought to strongly depend on basal ganglia functioning) and sequence learning (a motor task for which cerebellar functioning is essential) in eighteen patients with cervical dystonia and fifteen matched healthy controls.

Visuomotor learning was characterized by reduced activation in the basal ganglia (left putamen extending into the pallidum) in patients, while in controls frontal cortical activation (bilateral supplementary motor area extending into the premotor cortex) was more linked to task performance. These results underline basal ganglia dysfunction in idiopathic dystonia. During sequence learning, increased cerebellar activation was seen in patients (left cerebellar posterior lobe) and more linked to task performance (right cerebellar posterior lobe), pointing to a compensatory cerebellar role in idiopathic dystonia.

We also show an aberrant functional differentiation of these two subcortical structures to motor task demands in dystonia patients. These results indicate that cerebellar hyperactivity and reduced basal ganglia activation are not static traits in motor learning and control in idiopathic dystonia, but dynamic phenomena that can independently exist, depending on the functional demands of a motor task.

Introduction

Idiopathic dystonia is an invalidating hyperkinetic movement disorder characterized by involuntary muscle contractions, resulting in twisting movements, abnormal postures, or both. It is believed to be caused by abnormal functioning of brain regions as there is no structural brain lesion or degeneration in idiopathic dystonia. Both the basal ganglia and cerebellum are involved in the pathophysiology of this movement disorder, but their exact roles and their interaction remain undefined, making this a primary topic of interest in dystonia research [1-5].

Motor task-related cerebral abnormalities and/or deficient motor plans play a crucial role in dystonia. Symptoms of idiopathic focal dystonia are often restricted to specific, highly skilled movements (for example writer's cramp or musicians dystonia), acquired through extensive learning. Accordingly, studies have previously shown various forms of altered motor learning in patients with diverse forms of dystonia [6-12] and in accompanying animal models [13]. From a system-level perspective, motor learning is known to be supported by two complementary cerebral circuits, the cortico-striatal and the cortico-cerebellar loop [14, 15]. Here, we attempted to assess the balance, and the possible alteration thereof, between those two complementary circuits during motor learning in idiopathic focal cervical dystonia. We focus on two types of motor learning, visuomotor learning and sequence learning, known to rely predominantly on the cortico-striatal and the cortico-cerebellar loops, respectively. Visuomotor learning enables the formation of arbitrary associations between visual features of a stimulus and a specific motor action. For example, when learning to drive, we learn to associate the occurrence of a green traffic light with pressing the accelerator pedal. Human and macaque studies have shown that the basal ganglia play a crucial role in visuomotor learning, whereas the role of the cerebellum appears limited [16-18]. During motor sequence learning, a new series of movements is acquired in which the order and type of movements always remain identical. For example, to start your car you first put the key in the keyhole and then turn it. Both the basal ganglia and cerebellum are important for successful sequence learning, with the cerebellum being particularly involved in developing and retrieving internal models of a motor sequence [19-21].

Idiopathic dystonia has been most consistently linked to basal ganglia dysfunction [22]. Cerebellar abnormalities, increasingly observed in dystonia studies and repeatedly reviewed, are mostly explained by two opposing hypotheses. A dysfunctional cerebellum could be part of the aberrant sensorimotor network and a contributing factor in the clinical expression of dystonia. An alternative hypothesis suggests that the cerebellum partially compensates for deficient basal ganglia processing [1-5].

This experimental design allows us to distinguish between a number of possibilities on the functional and cerebral characteristics of idiopathic dystonia. If dystonia is primarily related to basal ganglia alterations, then learning-related activity in this structure should be particularly affected during visuomotor learning, given its reliance on the striatum [16-18].

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If dystonia is mitigated by compensatory activity in the cerebellum, then cerebellar activity should support learning-related performance, in particular during sequence learning [19-21].

Material and methods

Participants

Eighteen patients with cervical dystonia (mean age 57.7 ± 9.1, 7 male) and fifteen age- and sex-matched healthy controls (mean age 56.0 ± 9.3, 9 male) participated in this study, following approval by a local ethics committee (Committee on Research Involving Human Participants, region Arnhem-Nijmegen, The Netherlands). All participants gave written informed consent. One patient completed only the sequence-learning task, another patient completed only the visuomotor learning task, as they found participating in both tasks too strenuous. Patients were recruited from our outpatient movement disorder and botulinum toxin injection clinic, visiting the imaging centre once for both training and scanning. All patients received botulinum toxin treatment and were investigated in between treatments, e.g. when the effect of their last botulinum toxin injection was maximal. This was done to minimalize possible negative effects of head movement on task execution in patients. All patients responded favourably to the injections.

Experimental settings

Participants performed one training session (~30 min) and two scanning sessions (30 min each, one session for each of the two learning tasks). During both sessions, participants lay supine in a scanner bed. Visual stimuli were projected onto a screen behind the participant's head and were visible via a mirror attached to the head coil. An adjustable padded head holder was used to minimize head movements. Motor responses were recorded via an MR-compatible keypad (MRI devices, Waukesha, WI) that was positioned on the right side of the participants' abdomen with the four fingers (index, middle, ring, and little finger) of the right hand on the keypad. A PC running Presentation (Neurobehavioral Systems, San Fransisco, CA) was used to control stimulus presentation and response selection during the experiment.

Task

In the visuomotor learning task, participants were asked to learn (by trial and error) arbitrary associations between four randomly presented visual cues (line drawings of objects) and four motor responses (Figure 1A). Following presentation of a visual cue, participants had to select a response (within 4000 ms) by flexing one of four fingers of the right hand, pressing a button on a four-button keypad. After the motor response, visual feedback (a green or a red square) revealed whether the response was correct or incorrect (900 ms). A variable inter-trial duration was used (continuous uniform distribution between 1000-2000 ms).

In the sequence learning task, participants were asked to learn (by trial and error) an arbitrary sequence of four button presses. Following presentation of a visual cue (rotated line drawings), participants had to select a response (within 4000 ms). After the motor response, visual

feedback (a green or a red square) revealed whether the response was correct or incorrect (900 ms). In both visuomotor and sequence learning tasks, pseudorandomly interspersed with the visual cues triggering the participants responses, a number of inhibition cues (12.5% of trials) were also presented. Namely, when a line drawing of a hand was presented, participants were asked to widthdraw from responding in that trial. This procedure was introduced to ensure that participants identified each visual cue in both visuomotor and sequence learning tasks.

Procedure

During the training session, participants had to learn blocks of four different visuomotor associations and blocks of four different movements in a sequence. During the training session, the visuomotor and the sequence tasks were practiced over ten blocks each (learned blocks). The interval between the training session and the scanning session was approximately one hour.

During the scanning sessions, a learned block (consisting of either a four-associations set or a four-moves sequence learned during the training session) was alternated with a learning block (consisting of a new four-associations set or a new four-moves sequence). Between learned and learning blocks, there was a baseline interval of variable duration (10000-11000 ms, continuous uniform distribution). To warn that a block was coming up, a flashing star appeared on the screen (2000 ms). Participants randomly started training with either the sequence or the visuomotor task, but they always performed the scanning sessions in this same order, performing a minimum of 240 trials (120 learning trials and 120 learned trials) within the 30 min available.

Behavioural analysis

After removing missing trials and inhibition trials from each timeseries of trials per participant within each task, we created 8 equal bins, with 15 trials per bin, separately for learning and learned trials, corresponding to the minimum amount of finished trials. We considered mean reaction time and accuracy per bin.

A Bayesian statistical approach was used to analyse the behavioural data. Bayesian statistics provide a continuous measure of support of alternative and null hypotheses allowing an interpretation that does not depend on specific cut-off criteria.

A CONDITION (learning, learned) X BIN repeated measure Bayesian ANOVA with GROUP (controls, patients) as a between subject variable was conducted in Jasp (JASP TEAM Jasp version 0.7.5.6.) separately per task. The evidence (or lack) of a certain factor or interaction was indicated by the Bayes factor. The Bayes factor was quantified by the weighted likelihood ratio that compares the best fitting model for the data with the model that omits a particular factor (or interaction). For example, a Bayes factor of 5 indicates that the observed data are five times as likely to be consistent with the model that included a particular factor than the best model that does not include that factor. A Bayes factor can also state evidence against a
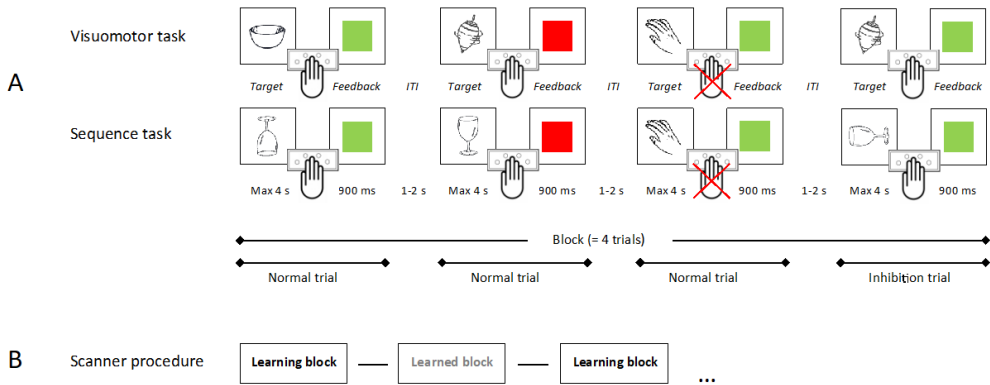
Figure 1 | Task set-up. A: Schematic display of visual targets for the visuomotor and sequence task. During normal trials, participants had 4000 ms to select their motor response on a four-button keypad after target presentation, to which the target disappeared and visual feedback was given (green square for correct and red square for incorrect motor response). A variable inter-trial duration was used (ITI 1000-2000 ms). During randomly appearing inhibition trials (in which the target consisted of a hand), participants were requested to withhold their motor response to which they also received feedback after target disappearance (green square for no motor response, red square for motor response). B: Schematic display of scanning session set-up. Learned blocks (consisting of four learned associations or one learned sequence introduced during the training session) were alternately presented with learning blocks (consisting of four new associations or one new sequence).

factor or evidence for invariance, in this case the winning model does not include the factor and the strength of evidence is again given by the comparison of the winning model to the best model with the factor included. We indicate the Bayes factor of the full model against a model that omits a factor with BF*factor*, a positive BFfactor (+) will state evidence in favor of inclusion of a factor, a negative BFfactor (-) will indicate evidence against a given factor.

In case of strong positive evidence for the inclusion of a factor or interaction, separate post-hoc Bayesian analysis for this factor or interaction were conducted using Bayesian independent T tests generating a Bayesian probablity of the alternative hypothesis occuring (BF_{10}). A BF_{10} of >30 is considered very strong evidence, BF_{10} 10-30 strong evidence, BF_{10} 3-10 moderate evidence and BF_{10} 1-3 anecdotal in favor of the alternative hypothesis. In general a BF_{10} of 3 or greater is considered to have the same value as a p<0.05.

Image acquisition

Images were acquired on a Siemens TRIO 3 Tesla MRI system (Siemens, Erlangen, Germany) equipped with echo planar imaging, using an 8-channel head coil for radio frequency transmission and signal reception.

First, 192 high resolution images were acquired using an T1-weighted three-dimensional magnetization-prepared rapid-acquisition gradient echo sequence (repetition time (TR) = 2300 ms; echo time (TE) = 3.03 ms; image matrix = 256 x 256 field of view (FOV), 256 mm; flip angle = 8°; slice thickness = 1.0 mm; voxel size = 1 x 1 x 1 mm).

Second, blood oxygen level-dependent (BOLD) sensitive functional images were acquired with a multi echo T2*-weighted echoplanar imaging sequence (TR = 2000 ms; TE1 = 6.9 ms; TE2 = 16.2 ms; TE3 = 24 ms; TE4 = 35 ms; TE5 = 44 ms; slice thickness = 3.0 mm; number of slices = 39; distance factor = 17%; image matrix = 64 x 64 field of view (FOV), 224 mm; flip angle = 80°; voxel size = 3.5 x 3.5 x 3 mm).

Image processing

All data were pre-processed and analysed with SMP8 (Statistic Parametric Mapping) (www.fil.ion.ucl.ac.uk/spm). First, the functional images were spatially realigned using a least squares approach and a 6 parameter (rigid body) spatial transformation. The time series of each voxel was then realigned temporally to acquisition of the first slice. Subsequently images were normalized to a standard EPI template centred in MNI space and resampled at an isotropic voxel size of 2 mm. The normalized images were smoothed with an isotropic 8-mm full-width-at-half-maximum Gaussian kernel. Finally, anatomical images were spatially coregistered to the mean of the functional images and spatially normalized using the same transformation matrix applied to the functional images.

Statistical model

The fMRI time series were analysed with an event-related approach in the context of a general linear model that, within each participant and task, considered the voxel-wise effect of six trial types (learning and learned trials, separately for correct, incorrect, and inhibition responses) and linear changes in those effects across scans (task X time interactions on correct and incorrect responses, both for learning and learned trials), for a total of ten regressor. This first-level model allows us to isolate, within each participant, cerebral effects that differ between learning state (overall differences between learning and learned trials), as well as between learning stage (time-varying differences between learning and learned trials across the scanning session). In the first-level model, a canonical hemodynamic response function (HRF) was convolved with the timeseries describing the onset of the six trial types (learning trial incorrect / learning trial correct / learned trial incorrect / learned trial correct / inhibition trial incorrect / inhibition trial correct), separately for each task (visuomotor, sequence) and for each patients and controls. The onset of the trial types was set on the appearance of the visual cue (inhibition correct trials), or on the response onset (other trial types), with a duration of 4900 ms (corresponding to the trial duration, for inhibition correct trials) or 900 ms (corresponding to the feedback duration, for the other trial types). Head motion effects were accounted for in the first-level model, considering linear, quadratic and cubic effects of the 6 parameters describing the motion of each volume, as well as the first derivative of those effects to control for spin-history.

In addition to the first-level model described above, focused on linear task X time interactions, we also considered an additional model focused on behaviorally-informed task X time interactions. Namely, instead of considering linear changes across scans between

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learning and learned trials, we considered changes as indexed by accuracy achieved by each participant within each block of four trials for each condition (percentage of correct trials per block, disregarding inhibition trials). Furthermore, instead of trial-specific events, this model considered a HRF time-locked to visual cue onset of the first trial, with a duration extending until end of feedback of the fourth (and last) trial of the block.

In the second level analysis, contrast images of correct learning and learned sequence and visuomotor trials were submitted to one sample (within group) or two sample (between group) T-tests.

Furthermore, contrast images of linear time-varying condition-related effects for each task were submitted to two sample T-tests to investigate brain regions that might react differentially over time during the two tasks. Finally, contrast images of the parametric effect of accuracy in learning and learned blocks were submitted to two sample T-tests to investigate brain regions that might differentially respond to accuracy level during the two tasks. Whole brain statistical maps were corrected for multiple comparisons (p<0.05, FWE-corrected) at the cluster level, on the basis of a cluster-forming threshold of $p < 0.01$ uncorrected.

ROI analysis

The main focus of this study was to characterize functioning of both the cerebellum and basal ganglia during the visuomotor and sequence task. Therefore, a region of interest (ROI) analysis was performed to further focus on differences in functioning of these regions between controls and patients during the two tasks. Percent signal change data were extracted for the bilateral basal ganglia and bilateral cerebellum using the MARSbar toolbox [23]. For all ROIs a sphere was drawn with a diameter of 8mm around the local maxima taken from an unbiased contrast. Bilateral basal ganglia coordinates (-20 -8 16 and 22 -6 14 for left and right respectively) were taken from the "visuomotor task > implicit baseline" contrast while bilateral cerebellum coordinates (32 -46 -28 and 32 -46 -28 for left and right respectively) were taken from the "sequence task > implicit baseline" contrast.

A TASK (sequence, visuomotor) × CONDITION (learning, learned) × REGION (left BG, right BG, left CER, right CER) repeated measure ANOVA with GROUP (controls, patients) as a between subject variable was conducted. To further characterize significant interaction effects, we performed repeated measures ANOVAs with the factors TASK × CONDITION as within subject factors and GROUP as between subject factor separately for each of our four regions. Lastly, a repeated measures ANOVAs with the factors TASK × CONDITION was done separately for each group.

Anatomical interference

When applicable, Brodmann areas were assigned on the basis of the SPM anatomy toolbox (SPM anatomy toolbox v1.8), i.e. the anatomical position of our significant clusters was formally tested against published three-dimensional probabilistic cytoarchitectonic maps.

Results

Behavioural effects - *Sequence task*

A CONDITION (learning, learned) X BIN repeated measure Bayesian ANOVA with GROUP (controls, patients) as a between subject variable was conducted. This resulted in a BF*condition* of +3.29X10^38, BF*bin* of + 9.42X10^29 and BF*bin x condition* of + 3.35X10^11. As shown in figure 2, there results indicate that the accuracy significantly increased over bins during the new learning condition, against a stable performance during the learned condition.

The Bayesian ANOVA generated evidence against the inclusion of BF*group*, BF*group x condition*, BF*group x bin* and BF*group x bin x condition* (BFgroup = -1.7, BFgroup x condition =-18.3, BFgroup x bin =-10.1, BF group x bin x condition =-89). These BFs indicate that both patients and control groups increased their accuracy over time in a comparable manner across conditions.

Behavioural effects -*Visuomotor task*

A CONDITION (learning, learned) X BIN repeated measure Bayesian ANOVA with GROUP (controls, patients) as a between subject variable was conducted. This resulted in a BF*condition* of +1.28X10^44. This means that it is 1.28X10^44 more likely that condition had a significant effect on the percentage correct responses than it did not. The BF*bin* and BF*bin x condition* were also strongly positive, +9.42X10^29 in favor of bin and +3.35X10^11 in favor of bin x condition. As shown in figure 2, these BFs indicate that accuracy increased over bins during the new learning condition, against a stable performance during the learned condition.

The BF*group* was +36 and BF*groups x condition* was +35. There was negative evidence in favor of inclusion of the interaction factor between *group* and *bin* (BFgroup x bin = -2.9) and *group x bin x condition* (BFgroup x bin x condition = -1.6). These BFs indicate that both patients and control groups increased their accuracy over time in a comparable manner across conditions. A post hoc Bayesian paired T-test for the mean percentage of correct responses resulted in a BF10 of 1.264 for the condition learning and a BF10 of 0.552 for the condition learned. Those BFs provide anecdotal evidence that patients have a lower percentage of correct responses during the condition learning (figure 2).

To summarize, we observed expected behavioural differences in accuracy scores and response times between learning versus learned trials for both tasks. Performance across
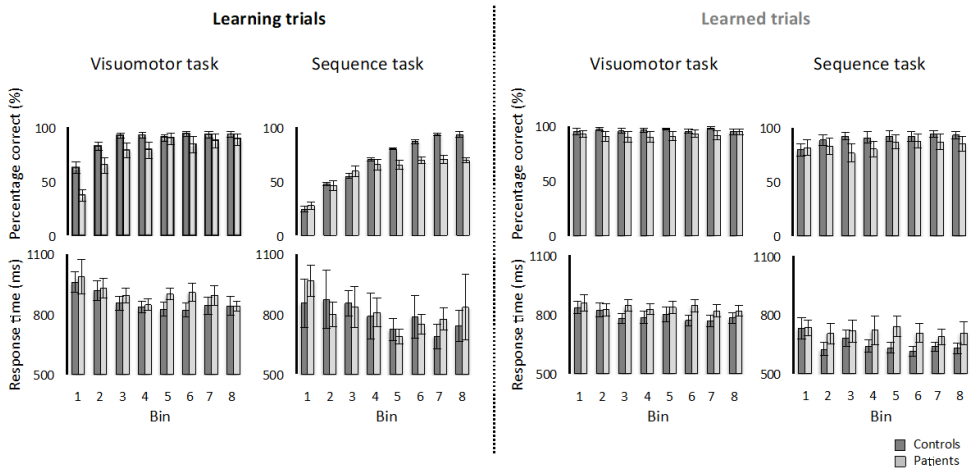
Figure 2 | Behavioural results. For each graph, average percentages of correct responses are shown per bin (consisting of 15 trials) with standard error of mean, for the visuomotor and sequence tasks. Left panels: task performance during learning trials. Right panels: task performance during learned trials.

learned trials was stable and matched across groups during both tasks. Performance during learning trials improved across groups during both tasks.

Imaging results - *Sequence task*

There was a between-groups difference driven by performance of the motor sequence task in the left cerebellar posterior lobe (learning correct trials, patients versus controls; -24, -74, -34; Z-value: 3.98, 1210 voxels, p_{FWE}=0.010), Figure 3. There was also a between-group learning-related difference in the right cerebellar posterior lobe extending into the left cerebellar posterior lobe (differential parametric modulation of accuracy between learning blocks, patients versus controls; 16, -68, -32; Z-value: 4.52, 1587 voxels, p_{FWE}=0.001; Figure 3). Namely, in the patients, cerebellar activity increased as a function of accuracy improvements, whereas it decreased in the controls. Reversed contrasts yielded no significant results.

Imaging results - *Visuomotor task*

There was a between-groups difference driven by performance of the visuomotor task in the left putamen (learning correct trials, controls versus patients; -24, 14, 12; Z-value: 3.44, 5763 voxels, p_{FWE}=0.006; Figure 3, based on a cluster-forming threshold of p<0.05). There was also a between-group learning-related difference in the bilateral supplementary motor area extending into the pre-motor cortex (differential parametric modulation of accuracy between learning blocks, controls versus patients; -18, -34, 62; Z-value 4.10, 3198 voxels, p_{FWE}<0.001; Figure 3). Namely, in controls, SMA/PMC activity increased as a function of accuracy improvements, wheras it decreased in patients. Reversed contrasts yielded no significant results.

Table 1 | Results of whole brain analysis. Whole brain statistical maps were corrected for multiple comparisons (FWE) on the cluster level with p <0.05, with uncorrected p < 0.01 used to generate statistical maps. Stereotactic coordinates are reported in Montreal Neurological Institute space.

Contrast	Laterality	Location	P-values	Voxels	Z-value	MNI coordinates
Patients > Controls sequence learning trials	Left	Cerebellum posterior lobe	0.010	1210	3.98	-24 -74 -34
Patients > Controls parametric modulation of accuracy sequence learning blocks	Right	Cerebellum posterior lobe	0.001	1587	4.52	16 -68 -32
Controls > Patients visuomotor learning trials	Left	Putamen	0.006	5763	3.44	-24 14 12
Controls > Patients parametric modulation of accuracy visuomotor learning blocks	Left	SMA	<0.001	3198	4.10	-18 -34 62
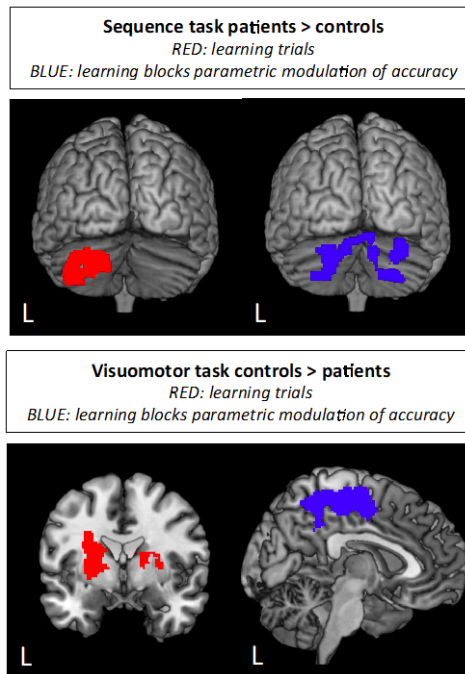
Figure 3 | Results of whole brain analysis. Whole brain statistical maps were corrected for multiple comparisons (FWE) on the cluster level with p <0.05. L indicates left side of the brain.

ROI analysis

To further investigate possible differences of basal ganglia and cerebellar functioning between patients and controls, especially their response to task (visuomotor vs. sequence task) and condition (learning vs. learned trials), we performed a ROI analysis.

For all ROIs, a sphere was drawn with a diameter of 8mm around the local maxima taken from an unbiased contrast. Bilateral basal ganglia coordinates were taken from the "visuomotor task > implicit baseline" contrast, while bilateral cerebellum coordinates were taken from the "sequence task > implicit baseline" contrast.

We will focus on the interaction effects that revealed significant post hoc group differences; the full ROI analysis are reported in supplementary methods.

Basal Ganglia

For the left basal ganglia, we found a significant main effect of CONDITION (p = .021), trend effects of TASK (p = .078) and GROUP (p =.063), and a significant interaction effect of TASK × GROUP (p = .01). The repeated measures ANOVAs with the factors TASK × CONDITION done separately for each group revealed that only in controls did the left basal ganglia show a differential activation for the two tasks (the visuomotor learning task induced more activation, p = .005) and that only in controls learning trials induced higher left basal ganglia activation as compared to learned trials (p = .029). Both effects were absent in the patient group (p = .531). For the right basal ganglia, we found a significant main effect of TASK (p = .034), CONDITION (p = .003), TASK × GROUP (p = .01) and TASK × CONDITION × GROUP (p =.048); no significant effect was observed for GROUP. The repeated measures ANOVAs with the factors TASK × CONDITION done separately for each group revealed that only in controls did the right basal ganglia show a difference in activation between the two tasks (the visuomotor learning task induced more activation, p = .004). This effect was absent in the patient group. In summary, only controls show a differentiation of basal ganglia functioning as a function of task (increased activation left and right basal ganglia for visuomotor task) and condition (increased left basal ganglia activation during learning trials) (figure 4).

Cerebellum

The right cerebellum showed a significant main effect of CONDITION (p = .002), with statistical trends in the interaction effects of TASK × GROUP (p = .052), and TASK × CONDITION × GROUP (p =.063). The repeated measures ANOVAs with the factors TASK × CONDITION done separately for each group revealed that only in controls learning trials induced higher right cerebellum activity as compared to learned trials (p = .002). This effect was absent in the patient group , so only controls show a differentiation of cerebellar functioning as a function of condition (increased activation of the right cerebellum during learning trials) (figure 4).
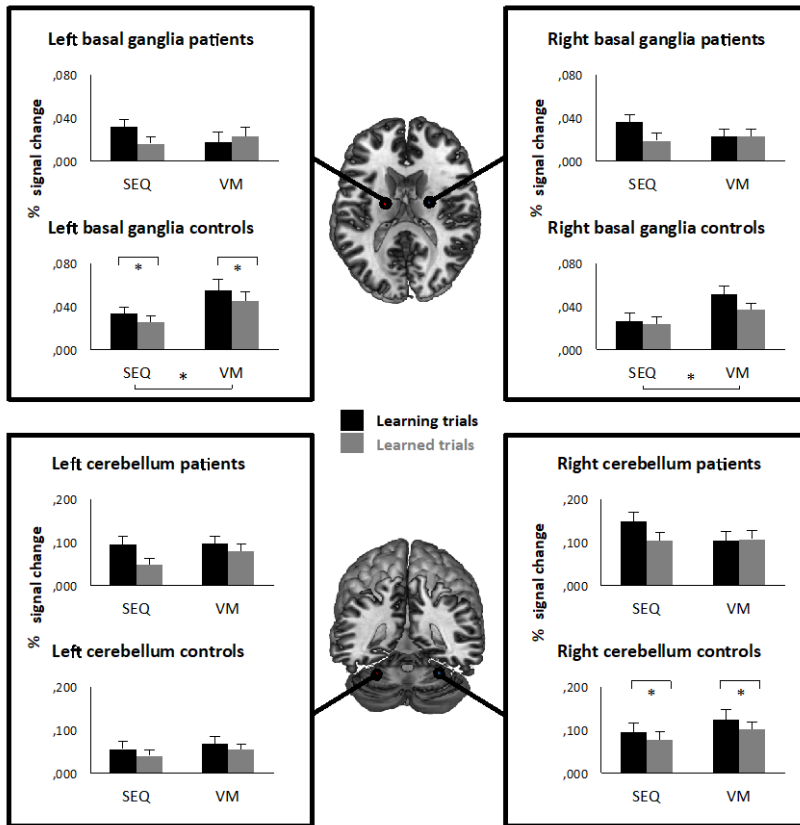
Figure 4 | ROI analysis. Percentual signal change of left and right basal ganglia and cerebellar activity in regions of interest separated per task (VM indicates visuomotor task, SEQ indicates sequence task), condition (learning trials depicted in black, learned trials depicted in grey) and group (controls and patients). Asterisks differences. For all ROIs, a sphere was drawn with a diameter of 8mm around the local maxima taken from an unbiased contrast.

Discussion

In this study, we investigated the basal ganglia and cerebellum, and their possible interaction, in the pathophysiology of idiopathic focal dystonia. Our aim was to assess a loss or gain of function in these subcortical structures by tracking the dynamics of their activity during two major forms of motor learning: sequence learning and visuomotor learning, known to rely mainly on cerebellar and basal ganglia circuits, respectively.

Basal ganglia dysfunction in idiopathic focal dystonia could be only demonstrated for tasks that rely highly on basal ganglia functioning, as we observed reduced activation of the left basal ganglia (putamen, extending into the pallidum) in patients during visuomotor learning, accompanied by minor behavioural impairments. No significant group differences in basal ganglia activation were observed during sequence learning. This is in line with findings of Carbon et al. who found increased cerebellar activation in DYT1 mutation carriers during

sequence learning but no differences in basal ganglia activation [11]. Abnormal putaminal activation and defective dopamine metabolism in the putamen have both been previously observed in writer's cramp (an other form of idiopathic focal dystonia) for different sensorimotor tasks [24, 25].

Further evidence suggesting a pathological basal ganglia activation pattern in patients comes from the observation that frontal cortical activation (bilateral supplementary motor area extending into the premotor cortex) was significantly more linked to performance in healthy controls during visuomotor learning than in patients. It is known that the putamen and PMC-SMA form a loop during motor learning that is considered to be inter-connected via distinct cortico-thalamic-basal ganglia loops [26, 27]. Thus, the observation that the left SMA/PMC was significantly less linked to performance in patients is probably a consequence from the reduced involvement of the left putamen during visuomotor learning.

Dresel et al. and Ibanez et al. studied functional MRI connectivity in writer's cramp using correlation analysis during rest and writing and observed reduced connectivity (indicated by reduced spatiotemporal correlations of BOLD signal fluctuations) in several nodes of the basal ganglia – thalamo – cortical network. These included reduced correlations between the left putamen and bilateral premotor cortical regions during right-handed writing and reduced correlations between the bilateral pallidum and left sensorimotor cortex during rest in patients with right-sided writer's cramp [28, 29]. Our study confirms dysfunction of basal ganglia –cortical loops during motor learning in dystonia.

During a motor task that relies more on intact cerebellar functioning (the sequence task), we observed no significant differences in behavioural performance of patients and controls. During sequence learning, patients did however show significantly increased left cerebellar activation compared to controls. In addition, only in patients was increased task performance linked to increased right cerebellar activation. The fact that in our study patients showed an increased association between cerebellar activation and task performance, supports the view that increased cerebellar activation in idiopathic focal dystonia could be compensatory in nature. However, we found no direct evidence of cerebellar compensation for basal ganglia dysfunction as no increased cerebellar activation was observed simultaneously with reduced basal ganglia activation in one and the same motor task.

Increased cerebellar activity has been repeatedly reported in previous patient studies and is often interpreted as a consequence of distorted cerebellar functioning.

The overflow of aberrant movement seen in dystonia is often linked to reduced motor cortical inhibition and increased plasticity observed in studies using transcranial magnetic stimulation (TMS). Hubsch et al. showed a reduced cerebellar influence on the modulation of sensorimotor plasticity in writer's cramp, which was inversely correlated with behavioural performance on a sensorimotor cerebellar adaptation task, i.e. better adaptation was associated with less cerebellar influence on motor cortical plasticity [12].

Cerebellar hyperactivity could result in the inability to modulate cortical excitability due to a ceiling effect in the study by Hubsch et al., or this could result from structural abnormalities of the cerebello-thalamo-cortical pathways, which were reported in patients with idiopathic dystonia and DYT gene carriers [30, 31]. The observation of Hubsch et al. that better adaptation was associated with less cerebellar influence on motor cortical plasticity seems to support the cerebellum being a (co-)driver in the clinical expression of dystonia.

Multiple animal studies also show that dystonia might arise through an abnormal increase in Purkinje cell firing or abnormal bursting patterns, rather than loss of cerebellar output [32, 33]. But while animal models are very important, findings cannot be directly extrapolated to humans. In addition, animal models often study dystonia due to a single gene defect or to an (induced) anatomic lesion, while in idiopathic types of focal dystonia neither are present. However, Neumann et al. recorded whole head magnetoencephalography simultaneously with pallidum activity in patients with idiopathic dystonia (cervical dystonia but also segmental and generalized dystonia) who underwent pallidal deep brain stimulation treatment. They observed a pallido-cerebellar source of alpha band coherence that showed an inverse correlation with dystonia severity [34]. Also in the previously mentioned study by Dresel et al., several cerebellar seed regions revealed a stronger negative functional connectivity to primary and secondary sensorimotor areas that decreased with greater dystonia severity and disease duration [28]. Thus, higher cerebellar connectivity was associated with better disease outcome in these studies, supporting our findings of a possibly compensatory cerebellar role in idiopathic dystonia.

In addition to impairments of basal ganglia functioning and compensatory cerebellar activation only being induced by specific motor task demands in dystonia, we found evidence for an aberrant functional differentiation of these two subcortical structures to motor task demands in patients. We observed that the level of basal ganglia and cerebellar activation in controls is significantly dependent on task (more basal ganglia activation during visuomotor task) and task load (more cerebellar and basal ganglia activation for learning than learned trials) whereas this differential activation was absent in patients.

We would like to shortly address the following study limitations. General findings are similar in the different analyses, even though some between group observations and site of cerebellar and/or basal ganglia findings differed between the analyses. This could have arisen from our relatively small sample size or the fact that the cerebellum and basal ganglia region used for the ROI analysis are dissimilar to the ones found in the whole-brain contrasts.

Delnooz et al. showed that connectivity abnormalities in cervical dystonia are partially normalized by botulinum toxin treatments, our patients were all examined in between treatments, when the effect was maximal, this could have influenced our results [35].

In this motor learning study, both aberrant cerebellar and basal ganglia performance was seen in patients with idiopathic focal dystonia, rather than isolated dysfunction. This supports the hypothesis that the underlying motor network in idiopathic dystonia involves both brain regions.

Whereas the existence of abnormal functioning in these two networks is probably interlinked during motor tasks, abnormalities were independently present.

Our study therefore proves that cerebellar hyperactivity and reduced basal ganglia activation are not a static abnormalities during motor performance in idiopathic dystonia, but more of a dynamic feature of which the presence depends on the task characteristics and demands. This complicates how we should assess the pathophysiology of this movement disorder and interpret study results.

Though we did not observe cerebellar hyperactivity together with reduced basal ganglia activation in the same motor task, our results point to a compensatory cerebellar role in idiopathic dystonia. Current advances in neuroscience methodology raise promising chances to further disentangle a compensatory versus disease related cerebellar engagement in idiopathic dystonia. However, it will remain difficult to interpret and integrate the results of various methods, also because results may vary depending on type of dystonia studied and compensatory cerebellar changes might still disrupt normal cerebellar functioning.

Our results question whether modifying cerebellar functioning, for example via non-invasive neuromodulation, should be therapeutically employed in idiopathic dystonia. Hyperactivity of the cerebellum seems to only partly accompany motor performance and appears beneficial to certain aspects of motor control.

Conclusion

Our two-task set-up of motor learning in cervical dystonia patients revealed minor behavioural impairments on visuomotor learning with reduced basal ganglia activity, and normal sequence learning performance paralleled by increased cerebellar activity. This supports the notion that both the basal ganglia and cerebellum contribute to the pathophysiology of this movement disorder. Increased cerebellar activation aided motor performance in dystonia patients, but we did not find direct evidence of cerebellar compensation of basal ganglia dysfunction (as the cerebellum did not 'jump in' during visuomotor learning).

Importantly, we showed that cerebellar hyperactivity and reduced basal ganglia activation are not static features of motor control and learning in idiopathic focal dystonia, but more dynamic phenomena. The aberrant functioning of the cerebellum and basal ganglia in dystonia patients resulted in the inability to adapt to the functional demands of a motor task. Our findings stress caution when making inferences on pathophysiological mechanisms in dystonia based on single-task experiments and question whether targeting the cerebellum could be therapeutically employed in idiopathic dystonia.

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Chapter 6

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Chapter 6

7.1

General discussion

This chapter begins with a concise list of new insights based on this thesis, followed by separate summaries of each of the previous chapters. In these summaries, I will briefly recapitulate the main methods and results. I shall emphasize the research questions that drove these studies and address the related new insights, placing them into the context of the current literature. Subsequently, I will suggest new avenues for future research in idiopathic focal dystonia. This chapter ends with the main conclusion of the work presented in this thesis.

New insights based on this thesis

- Patients with a combination of dystonia and cerebellar ataxia have a distinct neurophysiological pattern of cortical excitability compared to people with either isolated dystonia or isolated ataxia (chapter 3.1).

- Reduced motor intracortical inhibition is not always a prerequisite for cervical dystonia to occur (chapter 3.1).

- There is not a muscle-specific regulation of surround inhibition through cerebellar-brain-inhibition (CBI) at movement onset. There is a non-topographically specific modulation of CBI in association with movement initiation (chapter 3.2).

- Reduced surround inhibition in idiopathic focal dystonia is thus not (directly) caused by aberrant cerebellar-brain-inhibition (chapter 3.2).

- Eyeblink classical conditioning, a neurophysiological indicator of cerebellar dysfunction, is often abnormal in patients with idiopathic focal dystonia (chapter 4.2).

- Eyeblink classical conditioning (EBCC) can be improved in idiopathic cervical dystonia by practice (via repeated sessions of EBCC) and by direct non-invasive modulation of cerebellar excitability (through inhibitory cTBS). This indicates a (partly) functional and reversible disruption of cerebellar functioning in idiopathic cervical dystonia (chapter 4.2).

- Eyeblink classical conditioning is normal in patients with fixed dystonia, which argues against cerebellar dysfunction in this patient group (chapter 4.3).

- We demonstrated abnormal sensorimotor adaptation with the split-belt treadmill walking paradigm in patients with blepharospasm and writer's cramp, indicating that cerebellar dysfunction in idiopathic focal dystonia extends beyond more pure forms of cerebellum-dependent associative motor learning (chapter 5).

- A different abnormality, in degree and area, of cerebellar malfunctioning in various forms of idiopathic focal dystonia argues against uniform cerebellar pathology as the main driver in idiopathic focal dystonia (chapter 5).

- Increased cerebellar activation is beneficial to sequence learning performance in idiopathic cervical dystonia and could therefore serve as a compensatory mechanism (chapter 6).

- Aberrant functioning of the cerebellum and basal ganglia in idiopathic focal dystonia patients results in a reduced ability of these subcortical structures to adapt to the functional demands of a motor task (chapter 6).

- Cerebellar hyperactivity and reduced basal ganglia activation are not static features of motor control and learning in idiopathic focal dystonia, but dynamic phenomena that can individually exist depending on the functional demands of a motor task. The roles of the cerebellum and basal ganglia in dystonia are thus more complicated than simply a loss or gain of function (chapter 6).

Chapter 2
The cerebellum in dystonia – Help or hindrance?

My thesis started with a review in which I assessed the (at that point) existing literature providing evidence for a possible role of the cerebellum in the pathophysiology of dystonia. This review was an important stepping-stone to collect further ideas and set up the required experiments. Other groups have also started to review the existing evidence for a cerebellar role in the pathophysiology of dystonia and to form new hypotheses regarding this role, indicating an increasing interest in this topic [10-13].

What are the current main hypotheses on the role of the cerebellum in dystonia?
The basal ganglia and cerebellum play both important parts in motor control and their dysfunction can lead to diverse motor disorders. Whereas Parkinson's disease is historically considered to be a basal ganglia disorder and hereditary forms of ataxia are thought to be caused by cerebellar degeneration, dystonic movements are sometimes seen in both of these movement disorders, while acquired dystonia can occur due to both cerebellar and basal ganglia lesions [14, 15]. So, both these sites appear to have the capacity to (indirectly) induce dystonia. Anatomical studies in primates have now shown that direct subcortical communication between these two sites is present [16, 17].

Chen et al. recently presented evidence for cerebellar modulation of basal ganglia functioning, underlining the potential importance of this pathway in motor control but also

motor dysfunction [18]. At present, various hypotheses on cerebellar functioning in the dystonia network exist. Specifically, numerous studies propose that the cerebellum plays a primary role in the occurrence of dystonia, with elimination of dystonia after cerebellar lesions in animal models [19-22]. Altered cerebellar activity may cause altered basal ganglia activation, which in turn then causes dystonia. It is, however, unclear if and how these results in various rodent models can be extrapolated to humans, and if so, these are probably better applicable to the pathophysiology of hereditary dystonia. Clinically, the lack of traditional "cerebellar signs" in patients with idiopathic focal dystonia argues against the cerebellum being the site of primary pathology in these patients (although the question remains whether cerebellar signs can be assessed reliable in the face of marked dystonia in the same body parts). In addition, various cerebellar tasks are performed normally by patients with a form of idiopathic focal dystonia [3, 5, 7].

Another theory is that the cerebellum actually helps to maintain normal motor function in idiopathic focal dystonia by compensating for a dysfunctional motor network; the latter could be instigated by basal ganglia malfunction. Recent functional connectivity studies in patients with idiopathic focal dystonia support this assumption by showing increased cerebellar connectivity with basal ganglia and/or sensorimotor cortical areas that is associated with reduced dystonic symptom severity [23, 24].
The cerebellum could also only play a compensatory role in the motor network during the preclinical and earlier disease stages, whereas with disease progression, these compensatory mechanisms might fail and even become co-pathogenetic in more advanced disease.
Findings of impaired performance on cerebellar based tasks or reduced cerebellar priming of cortical excitability/plasticity do not directly support one of the above theories as compensatory changes in the cerebellum may also disrupt normal cerebellar functioning.

Abnormalities observed in the cerebellum might also purely be a secondary consequence with limited pathophysiological relevance; for example, secondary to other motor network (i.e. basal ganglia) dysfunction, to aberrant sensory input, or to abnormal body posturing in idiopathic focal dystonia, or simply as co-existent lesion (many neurodegenerative disorders are multifocal in nature). But observations of cerebellar performance being linked to behavioural and/or clinical parameters in both dystonia animal models and patient studies strongly argue against this [7, 8, 19-26].
There appears to be consensus on a cerebellar contribution to the pathophysiology of dystonia, but more work is needed to better define the role of the cerebellum in the various manifestations of this movement disorder.

Chapter 3.1
A distinctive pattern of cortical excitability in patients with the syndrome of dystonia and cerebellar ataxia.
Chapter 3.2
Cerebellar brain inhibition is decreased in active and surround muscles at the onset of voluntary movement.

Neurophysiological studies investigating dystonia have revealed various motor cortical abnormalities in this movement disorder. In chapter 3.1 and 3.2 I used this groundwork to investigate a possible role for the cerebellum in the generation of these abnormalities. I will also discuss several subsequent studies that have pursued this goal. These studies have evolved from the recognition of cerebellar priming of motor cortical excitability in idiopathic dystonia to investigating the clinical relevance; and aiming to normalise motor cortical abnormalities by cerebellar stimulation. See box 1 of the introduction chapter for further information regarding the TMS methods used in these studies.

The motor cortical excitability profile of patients with dystonia; is there a relationship between observed abnormalities and clinical phenotype?
In chapter 3.1, I investigated patients with the rare syndrome of cervical dystonia and cerebellar ataxia (DYTCA). DYTCA patients show generally prominent and disabling focal dystonia with milder slowly progressive clinical "cerebellar signs" (ataxia). Kuoppamaki and van de Warrenburg have reported series of patients with this syndrome [27, 28]. The etiology of DYTCA is underdetermined but probably heterogeneous and largely genetic. Recently, Doss et al. described mutations in COX20 (FAM36A) as a novel cause of a recessively inherited, early-onset dystonia-ataxia syndrome (DYTCA) [29].

I investigated if aberrant cerebellar functioning of DYTCA patients is influencing their motor cortical excitability profile, or if their cortical excitability profile is similar to that of patients with "pure" idiopathic focal dystonia. This knowledge is also relevant to justify previous links between excitability abnormalities of the motor cortex and dystonia [30]. The cortical excitability profiles of DYTCA patients, idiopathic focal dystonia patients and healthy controls were examined in this study.

A distinctive pattern of cortical excitability in DYTCA patients was identified: hyperexcitable short-interval intracortical inhibition (SICI), contrasting findings in idiopathic focal dystonia. Several forms of reduced inhibition of the motor cortex have previously been observed in electrophysiological studies of patients with idiopathic dystonia [31-34]. The fact that the cortical excitability profile in patients with this DYTCA syndrome reflected increased inhibition rather than reduced inhibition is therefore surprising, given that the latter would be expected in idiopathic focal dystonia. A more limited number of studies have assessed the

motor cortical excitability profile of patients with cerebellar lesions. The balance between cortical excitatory and inhibitory circuitry appears disturbed in such patients; however, the shift seems to be opposite to that seen in dystonia [35-40]. These data cautiously suggest that the cortical excitability profile found in DYTCA patients could perhaps be attributed to the cerebellar involvement in this movement disorder, as a reflection of abnormal cerebellar modulation of the functional connections in the motor cortex in this patient group.

Chapter 3.1 also advocates that reduced motor intracortical inhibition is not a pre-requisite for idiopathic dystonia to occur. This is supported by various other studies. Normal short-interval intracortical inhibition (SICI) has earlier been observed in patients with idiopathic dystonia [41, 42]. In contrast, reduced SICI can also be found in fixed dystonia, non-manifesting DYT1 mutation carriers and patients with acquired dystonia due to basal ganglia and/or thalamic lesions [43, 44].

Whereas reduced SICI seems non-specific to idiopathic dystonia, Kojovic et al. hypothesized that abnormally enhanced motor cortex plasticity is an endophenotypic trait more specific to idiopathic dystonia, as motor cortex plasticity is normal in acquired and fixed dystonia [44, 45].

Could cerebellar modulation by rTMS normalize sensorimotor cortical excitability in idiopathic focal dystonia?

Increased cortical plasticity in idiopathic dystonia is reflected by an enhanced response to PAS (paired associative stimulation) in patients with idiopathic dystonia [44, 46]. PAS is a protocol that combines peripheral sensory stimulation with transcranial magnetic stimulation (TMS) over the contralateral motor cortex, resulting in plastic changes of excitability in the human motor cortex.

Given that the effect of a motor cortex PAS protocol is modifiable by cerebellar repetitive transcranial magnetic stimulation in healthy controls, various groups tested if they could use the same methods to normalize the increased sensorimotor cortical plasticity in idiopathic focal dystonia [7, 25, 47]. Koch et al. found that two weeks of (inhibitory) cerebellar continuous theta burst stimulation in patients with cervical dystonia normalized the pattern of topographically specific induced plasticity tested by a PAS25 protocol. [25]. Their results oppose those of Sadnicka et al. who observed that a single session of (excitatory) cerebellar anodal transcranial direct current stimulation did not affect the response to a PAS25 protocol in writer´s cramp patients. They also noticed that an increased response to PAS25 is not observable in all writer's cramp patients [47]. The results of of Hubsch et al. are consistent with those of Sadnicka et al. They showed that patients with writer's cramp as a group have lost the normal bidirectional cerebellar priming effect on M1 sensorimotor plasticity. [7]. Taken together, more studies are necessary to confirm that normalizing sensorimotor cortical

plasticity can be achieved by cerebellar modulation and to investigate whether positive findings are paradigm (i.e. number of sessions, excitatory versus inhibitory stimulation, rTMS versus tDCS, site of stimulation) or patient (i.e. site of focal dystonia, disease duration, severity of symptoms) specific.

Could cerebellar brain inhibition aid surround inhibition?

I investigated the cerebellar capacity to selectively model motor cortical activity in chapter 3.2, by investigating a possible relationship between two phenomena observed to be deficient in idiopathic focal dystonia: surround inhibition (SI) and cerebellar brain inhibition (CBI).

Highly selective activation of desired muscles for a movement and inhibition of adjacent muscles is attributed to a phenomenon referred to as motor surround inhibition (SI). SI differentially modulates corticospinal excitability in active and surrounding muscles, demonstrated by suppression of motor evoked potentials (MEPs) of the surrounding muscles by TMS pulses. SI is reduced in patients with idiopathic focal dystonia during movement onset, which could lead to the excessive co-contractions of muscles characterizing this movement disorder [48, 49]. Various studies have investigated the underlying (sub)cortical circuits that could contribute to deficient SI in idiopathic focal dystonia [50-52]. I investigated a possible cerebellar role in the genesis of SI.

In a TMS protocol called cerebellar brain inhibition (CBI), a cerebellar transcranial magnetic pulse is thought to activate the Purkinje cells which in turn inhibit the dendate nucleus, thereby inhibiting the tonic dendate-thalamo-cortical facilitatory drive. So, during CBI, a conditioning cerebellar stimulus reduces the motor evoked potential (MEP) of the shortly followed contralateral motor cortex stimulus. Cerebellar brain inhibition (CBI) is known to be active at rest and during tonic muscle contraction in healthy participants [53-55].
Brighina et al. found that CBI is reduced in focal hand dystonia, stressing our hypothesis that aberrant CBI might aid reduced SI in idiopathic dystonia. [42] Bradnam et al. observed normal CBI in these patients using different methods; however, greater CBI was associated with worse hand function, suggesting a possible clinical relevance of CBI in dystonia [56]. Koch et al. also found no significant differences between CBI in patients with cervical dystonia and controls [25].

I investigated if surround inhibition (SI) could be modulated by cerebellar brain inhibition (CBI) at movement onset in healthy participants. CBI was, however, reduced at movement onset for all studied muscles. My findings could thus not directly link SI functionally with CBI at movement onset, but they do indicate a non-topographically specific modulation of CBI in association with initiation of voluntary movement. This could render the motor cortex to be more sensitive to other cortical and/or subcortical (possibly basal ganglia) influences at movement onset.

Chapter 7.1

CBI might have a different role in selective *on-going* muscle activation. Panyakaew et al. recently found that during selective ongoing tonic muscle contraction (in which SI is absent) there is specifically reduced CBI for the activated target muscle [57]. The authors hypothesized that this might be important for shaping motor programs during forms of error-driven motor learning. After split-belt adaptation (walking on a treadmill where the left and right leg can be forced to move with different speeds), a form of feedforward motor learning, Jayaram et al. did observe that CBI decreased proportionally to the magnitude of adaptation in healthy subjects [58].

Chapter 4.1
Cerebellar theta burst inhibition impairs eyeblink classical conditioning.
Chapter 4.2
Cerebellum-dependent associative learning deficits in primary dystonia are normalized by rTMS and practise.
Chapter 4.3
Normal eyeblink classical conditioning in patients with fixed dystonia.

Some of the first evidence of aberrant cerebellar functioning in dystonia comes from Teo et al. (2009) who found eyeblink classical conditioning (EBCC) to be impaired in patients with idiopathic focal dystonia [1]. EBCC is a protocol of associative motor learning in which paired presentation of a conditioned (CS) and unconditioned stimulus (US) leads to the production of a conditioned eyeblink response (CR). The anatomical substrate for EBCC is the cerebellum. For my studies of chapter 4, I used EBCC as a neurophysiological indicator of cerebellar dysfunction. See box 2 of the introduction chapter for more information on EBCC.

Are abnormalities in cerebellar functioning, reflected by aberrant EBCC, seen in all forms of dystonia?
Previous studies in idiopathic focal dystonia have shown that earlier electrophysiological abnormalities observed in this movement disorder proved to be non-specific for this patient group and could for example result from deficient sensory feedback due to aberrant body posturing. In chapter 4.3 I therefore studied EBCC in patients with fixed dystonia and observed that there were no EBCC impairments in non-medicated patients with fixed dystonia. This argues against abnormal cerebellar functioning in this patient group and suggests that impairments in cerebellar functioning are specific for the pathophysiology of idiopathic focal dystonia. Patients with acquired dystonia due to thalamic or basal ganglia lesions also show normal eyeblink classical conditioning [44]. Very recently Antelmi et al. reported a difference in eyeblink classical conditioning capacity between dystonic patients with and without tremor, indicating that aberrant cerebellar functioning in dystonia might be

tremor dependent, or that cerebellar involvement contributes to the variant phenotype with tremor [59].

Is cerebellar functioning as measured by EBCC modifiable by rTMS over the cerebellum in healthy controls and patients with idiopathic focal dystonia?

In chapter 4.1 I examined whether cerebellar functioning is temporarily modifiable by cerebellar theta burst stimulation (cTBS) in healthy subjects. I did this by studying the acquisition and retention of EBCC in healthy volunteers after cTBS over the right cerebellar hemisphere. Cerebellar cTBS disrupted the acquisition of conditioned eyeblink responses in healthy volunteers.

Knowing that cerebellar functioning is modifiable by cTBS in healthy controls, I investigated EBCC and the effect of cerebellar cTBS on EBCC more extensively in patients with idiopathic dystonia in chapter 4.2. Patients with focal dystonia showed an *improvement* of EBCC in a second session, which is in contrast to patients with proven cerebellar pathology who do not show further improvement of EBCC in additional sessions [60-62]. Interestingly, cerebellar cTBS paradoxically *normalized* EBCC in patients with CD. This points to a functional and reversible disruption of the cerebellum in dystonia.

The explanation for the contrasting results of cerebellar stimulation in patients and controls is as yet unclear. One obvious explanation relates to differences in either patient characteristics (even the group of patients with idiopathic dystonia presumably includes a heteregenous mix of underlying diagnoses) or methodologies used. One other possibility is that cerebellar cTBS normalizes aberrant cerebellar activity in patients, which is supported by the fact that imaging studies in dystonia have frequently reported hyperactivity of the cerebellum. This could indicate cerebellar compensation or be a sign of cerebellar recruitment in the abnormal sensorimotor network. Nevertheless, these results reinforce that reduced EBCC in dystonia patients is indeed a reflection of aberrant cerebellar functioning, and that this is not due to static, structural cerebellar pathology.

If altering cerebellar functioning in cervical dystonia patients by inhibitory rTMS has positive results on EBCC and cerebellar stimulation might have the capacity to normalise sensorimotor cortical excitability in dystonia; studying clinical consequences of cerebellar stimulation with rTMS in focal dystonia seems a logical next step. Keeping in mind that aberrant cerebellar functioning could be detrimental for pure cerebellar based tasks such as EBCC, it might be compensatory in the bigger dystonia sensorimotor network defect.

Could rTMS over the cerebellum be therapeutically employed in idiopathic focal dystonia?

Various authors examined if changing cerebellar functioning with rTMS could be therapeutically employed in idiopathic dystonia. Both positive and negative findings were reported. First, Sadnicka et al. investigated the effects of (excitatory) anodal cerebellar transcranial direct current stimulation versus sham stimulation in ten patients with writer's cramp. They failed to reduce dystonia symptoms by cerebellar excitatory stimulation in dystonia [47]. Second, Bologna et al. did not observe any effect of a single cerebellar cTBS session on movement kinematics recorded with infrared cameras or clinical rating scales in patients with focal hand dystonia or with cervical dystonia [63]. In our own group, Linssen et al. (2014) tested the effects of cerebellar (inhibitory) continuous theta burst stimulation versus sham stimulation in ten patients with writer's cramp. We also observed no significant effect on writing performance following cerebellar inhibitory stimulation [64].

However, Bradnam et al. did observe a positive effect of anodal cerebellar transcranial direct current stimulation on recorded handwriting and cycle drawing kinematics of focal hand dystonia patients, whereas cathodal (inhibitory) cerebellar transcranial direct current stimulation evoked similar responses to handwriting but not cycle drawing [56]. More recently, Koch et al. investigated the effect of two weeks of inhibitory cerebellar (inhibitory) continuous theta burst stimulation versus sham stimulation in twenty patients with cervical dystonia [25]. They also documented a modest improvement of dystonic symptoms measured by a dystonia rating scale specific for cervical dystonia. Measurements using a more generalized dystonia scale did not improve. Clinical results were however transient as they faded within two weeks after the last session of cerebellar stimulation. As we stated earlier, these conflicting results summarized above could have resulted from different methods used and the different types of focal dystonia studied.

The findings of Koch et al. and Bradnam et al. are certainly hopeful and encourage us to further investigate the possibility of a clinical application of cerebellar stimulation in patients with idiopathic dystonia. It must be noticed, however, that the observed clinical improvement was only minor and transient, and others have not yet reproduced these results. Just to illustrate the long trajectory from "bench to bedside" for these types of novel interventions: although not fully comparable, clinical improvement after applying inhibitory repetitive transcranial magnetic stimulation (rTMS) over the premotor cortex in patients with writer's cramp was first reported in 2005, but now, rTMS over the premotor cortex is still a research intervention and is not yet used as a treatment option in idiopathic focal dystonia [65].
Better understanding of the role of the cerebellum in dystonia may still be the essential first step necessary before evaluating cerebellar stimulation as a treatment option for dystonia.

Chapter 5
A gait paradigm reveals different patterns of abnormal cerebellar motor learning in primary focal dystonias.

Is aberrant cerebellar functioning also affecting more complex forms of error-driven sensorimotor learning in idiopathic focal dystonia?

The cerebellum has been hypothesized to contribute to sensorimotor control by a forward model. In this model, motor output is modified by error signals that reflect the difference between expected and observed sensory cerebellar input [66]. The cerebellum uses this system to update subsequent motor performance during a motor adaptation task. Previous studies have shown that cerebellar damage indeed leads to deficits in various sensorimotor adaptation tasks [66-68].

Motor adaptation was tested in patients with cervical dystonia, blepharospasm and writer's cramp in chapter 5 by investigating split-belt walking. During this task, participants are asked to adjust to a new type of walking pattern on a treadmill with various speeds for each leg. Two types of gait adjustments are seen during split-belt walking: 1) direct reactive adjustments of walking parameters (e.g. stride length and time in stance) to accommodate the novel difference in belt speeds, and 2) adaptive feedforward adjustments in step length, time in double support, oscillation and phasing parameters [69]. For more information on split-belt walking, I refer to box 3 of the introduction chapter.

Patients with blepharospasm and writer's cramp showed impaired adaptive feedforward adjustments of their split-belt walking pattern. Both patient groups showed slower speed of step length symmetry adaptation. The speed of step length symmetry adaptation can be influenced by changing both spatial and temporal parameters of walking. Patients with writer's cramp were impaired in temporal parameters of walking adaptation (double support time, phasing) whereas patients with blepharospasm showed abnormalities in both spatial (oscillation) and temporal parameters (double support time) of adaptation. In contrast, patients with cervical dystonia showed an adaptation pattern similar to healthy controls.
This reinforces the current concept of cerebellar dysfunction in idiopathic focal dystonia and that this extends beyond more pure forms of cerebellum-dependent motor learning paradigms (such as EBCC in chapter 4).

What have we learned from other dystonia studies investigating cerebellar dependent behavioural tasks?

Our split-belt study also showed that patients with various forms of idiopathic focal dystonia show different degrees of aberrant cerebellar functioning.
The following table is a condens overview of other studies investigating various tasks known to be strongly cerebellum dependent in different dystonia patient groups.

Table 1 | Overview of studies investigating cerebellum-dependent task in dystonia patients.

Study	Patient group	Cerebellar task	Main outcome
Teo et al. 2009 [1]	12 patients with adult onset focal dystonia (7 cervical dystonia, 5 task specific hand dystonia).	Eyeblink classical conditioning.	Acquisition of EBCC impaired in dystonia patients, timing of conditioned responses intact.
Antelmi et a. 2016	25 patients with idiopathic isolated cervical dystonia, with and without tremor.	Eyeblink classical conditioning.	Acquisition of EBCC selectively impaired in dystonia patients with tremor.
Hoffland et al. 2013 [2]	19 patients with idiopathic cervical dystonia.	Eyeblink classical conditioning.	Acquisition of EBCC impaired in dystonia patients, timing of conditioned responses intact. Improvement of EBCC over subsequent sessions and after cerebellar cTBS in patients.
Sadnicka et al. 2013 [3]	20 patients with idiopathic cervical dystonia. NB 12 patients head tremor.	Motor adaptation task with right arm.	Rates of adaptation identical to healthy controls.
Sadnicka et al. 2014 [4]	11 DYT1 and 5 DYT6 dystonia patients.	Eyeblink classical conditioning.	Acquisition of EBCC identical to healthy controls.
Katschnig-Winter et al. 2013 [5]	12 patients with cervical dystonia. NB: No head tremor or segmental spread of dystonia towards hands.	Motor adaptation and sequence learning with dominant right arm.	Rates of adaptation and sequence learning similar to healthy controls, patients possibly employ a different task strategy during sequence learning.
Hubsch et al. 2011 [6]	14 patients with DYT11 dystonia ("myoclonus dystonia").	Adaptation of saccadic eye movements.	Rates of adaptation impaired compared to healthy controls.
Hubsch et al. 2013 [7]	21 patients with writer's cramp.	Motor adaptation task with dominant right arm.	Rates of adaptation identical to healthy controls, impaired washing out of after-effects.
Carbon et al. 2008 [8]	6 non manifesting DYT1 carriers.	Sequence learning with dominant right arm (PET study).	Sequence learning impaired with increased contralateral cerebellar activation.
Carbon et al. 2011 [9]	19 DYT1 carriers (10 non manifesting, 9 manifesting) and 11 DYT6 carriers (4 non manifesting, 7 manifesting).	Sequence learning with dominant right arm (PET study)	Sequence learning impaired with increased contralateral cerebellar activation in (non manifesting and manifesting) DYT1 carriers. Normal sequence learning in DYT6 carriers.

Noticeably, contrasting findings have been observed in these studies. Firstly, we see opposing findings when testing the same cerebellar task in different patient groups. One might suggest that this could be explained by methodological differences between studies. However, even in single studies (our own split-belt study, and Carbon et al. 2011 [9]) there are opposing results. There are not only differences between forms of dystonia with a clear genetic origin versus idiopathic focal dystonia, but even between different types of focal idiopathic dystonia. The role of the cerebellum in the pathophysiology of dystonia might therefore vary depending on dystonia subtype. This could result from purer neuroanatomical changes associated with a certain genetic background. Other possible explanations are differences in site of dystonia symptoms, accompanying tremor, disease duration or events that may have triggered dystonia (such as movement repetition in task-specific dystonias). Also, when looking at the results we see that in the same dystonia subtype group, different cerebellar tasks yield contrasting results of impairment. Apparently, the regions of the cerebellum engaged in the task studied and involvement of other brain regions or networks in that task determine which (level of) behavioural abnormalities are observed. This makes it difficult to define the extent and nature of cerebellar involvement in patients with dystonia. These results do challenge the concept of a simple loss or gain of cerebellar functioning in dystonia. It also emphasizes that drawing major conclusions based on individual studies of behavioural abnormalities on cerebellar-based tasks in dystonia patients is not justified.

Chapter 6
Cerebellar and basal ganglia contributions to motor learning in idiopathic focal dystonia

Cerebellar abnormalities in the studies mentioned are most often explained by the following two hypotheses: 1) either a dysfunctional cerebellum is driving or is part of the aberrant sensorimotor network in dystonia, or 2) a hyperactive cerebellum might compensate for other deficient circuits or brain areas such as the basal ganglia. In chapter 6, I therefore further investigated how the basal ganglia and cerebellum interact in this movement disorder in an fMRI set up. For more information on fMRI, I refer to box 4 of the introduction chapter.

Could the cerebellum compensate for basal ganglia impairments in idiopathic cervical dystonia and if so are there boundary conditions for this compensatory activity?
I investigated two different forms of motor learning in cervical dystonia: sequence learning and visuomotor learning. During sequence learning, a new series of movements is acquired in which the order and type of movements always remains identical. For example, to start your car, you first put the key in the keyhole and then turn it. During visuomotor learning the non-spatial visual features of a stimulus are connected to a specific motor action. For example, when learning to drive we associate the occurrence of a green traffic light with pressing the

throttle. Note that this is different from formerly discussed visuomotor adaptation, which requires individuals to adjust spatial, goal-directed movements to distorted visual feedback.

The cerebral networks activated during these two motor tasks are distinct with a differential emphasis on basal ganglia and cerebellar functioning. The basal ganglia are more engaged in visuomotor learning, whereas sequence learning relies heavily on the cerebellum [70-75]. We used the distinct features of these tasks to investigate cerebellar and basal ganglia functioning in motor control of idiopathic focal dystonia patients. The two motor learning tasks were performed with the right hand by patients with cervical dystonia and healthy controls and kept as similar as possible in execution to ensure that observed differences were solely related to task. Patients showed reduced basal ganglia activation (left putamen plus pallidum) during visuomotor learning, and frontal cortical activation (bilateral SMA/PMC) was less related to task performance. Patients showed increased cerebellar activation (left cerebellum posterior lobe) during sequence learning, and cerebellar activation (right cerebellum posterior lobe extending into left cerebellum posterior lobe) was more related to task performance. This cautiously suggests that impairments of the basal ganglia-cortical network are elicited during motor tasks that put a high emphasis on basal ganglia performance (visuomotor learning), as a motor task less dependent on basal ganglia functioning (sequence learning) does not reveal abnormalities. The cerebellum might play a compensatory role in certain aspects of motor control in dystonia, but only during motor tasks that are strongly cerebellar dependent (sequence learning). These two combined observations could argue for direct cerebellar compensation of basal ganglia dysfunction, but I did not observe that the cerebellum "jumped in" for the basal ganglia during the visuomotor task.

This study examines possible consequences of baseline aberrant functional connectivity of the basal ganglia and cerebellum in the motor network to task performance. Previous studies have investigated functional connectivity in patients with idiopathic dystonia by spatio-temporal coupling of spontaneous fluctuations of brain activity in rest. In line with my findings, these studies reported reduced functional basal ganglia connectivity, including 1) reduced connectivity between the putamen or pallidum with premotor cortical regions, 2) increased functional cerebellar connectivity, such as increased connectivity between the cerebellum and pallidum, and between the cerebellum and primary plus secondary cortical sensorimotor areas [23, 24]. Increased cerebellar connectivity was positively associated with reduced dystonic sympton severity [23, 24]. My results cautiously support the assumption that abnormalities of basal ganglia and cerebellar functioning are not static and not solely related to motor execution, but are rather dynamic and dependent on the context of motor performance. Note that symptoms of dystonia are also often task specific. These findings also indicate the complexity of investigations of the role of the cerebellum in this movement disorder.

Suggestions for future research

My thesis has focused on the cerebellar role in the pathological sensorimotor network of idiopathic focal dystonia. But it must be noticed that even the role of the cerebellum in the physiological sensorimotor network is still not fully clarified. In this section, I discuss new approaches to investigate the role of the cerebellum in the pathophysiology of dystonia as well as in normal motor functioning.

I. Previous research, including my own, has identified various neurophysiological (cerebellar) abnormalities in (family members of) patients with idiopathic and acquired dystonia. Increased knowledge might be gained from more widespread use of these study methods. Given the neurophysiological differences between idiopathic and acquired dystonia due to non-cerebellar lesions, it would be interesting to study cortical excitability and (cerebellar priming of) sensorimotor plasticity in patients with acquired dystonia due to cerebellar lesions to see if these correspond to the abnormalities found in patients with DYTCA (hyperexcitable SICI) or idiopathic focal dystonia patients (reduced SICI and impaired sensorimotor plasticity) [32, 43, 76, 77].

Idiopathic focal dystonia does not have a clear Mendelian pattern of inheritance but familial occurrences of idiopathic focal dystonia do indicate a genetic susceptibility. Abnormal temporal discrimination has for example been reported in unaffected first-degree relatives of idiopathic dystonia patients [78]. It would be valuable to see whether (clinical and neurophysiological) indicators of cerebellar dysfunction also exist in these relatives, such as aberrant eyeblink classical conditioning (EBCC), cerebellar-brain-inhibition (CBI), or aberrant cerebellar priming of sensorimotor plasticity. This might then possibly clarify whether cerebellar dysfunction in idiopathic focal dystonia is a primary abnormality or only occurs secondary to disease manifestation, and whether this could serve as an endophenotypic trait. In addition, one could then test in unaffected first-degree relatives whether these clinical and/or neurophysiological abnormalities show a correlation with these previously observed abnormalities in temporal discrimination.

II. Most of the current TMS studies investigating cortical excitability and the cerebellar modulation thereof, including my own, have been executed at rest. More valuable information could possibly be obtained from studies focusing on movement (preparation) and/or task performance in dystonia.

In a yet to be published study, I found non-selective muscle excitation before movement onset in task-specific dystonia (writer's cramp) that was preceded by non-selective muscle inhibition during the *pre-cue phase* of motor preparation. This implies that reduced inhibition is only one aspect of a more generalized inability to correctly select and construct a movement.

TMS studies have focused on cerebellar priming of sensorimotor cortical plasticity during rest and/or reviewed the correlation thereof with visuomotor adaptation performance in

idiopathic dystonia [7, 25, 47]. However, changes in motor corticospinal excitability and cerebellar-M1 connectivity have been reported *after a motor adaptation task* in healthy participants [58, 79]. Motor corticospinal excitability, cerebellar-M1 connectivity, and/ or sensorimotor plasticity could be investigated in patients with idiopathic dystonia and compared with healthy controls *after* a cerebellar learning task to study possible real life consequences of aberrant cerebellar priming of the motor cortex in dystonia patients. Our previously discussed cerebellar-brain-inhibition (CBI) findings compared with those of Panyakaew et al. also suggest that it might be interesting to further explore a possible *time course* in the modulation of CBI during the preparation and execution phases of movement in healthy controls and patients with cervical dystonia.

In chapter 6, I observed abnormalities of basal ganglia and cerebellar functioning specific to the context of a motor task in cervical dystonia. Idiopathic focal dystonia can affect different body parts but can also be task specific. Interestingly, Choi and Bastian observed that walking adaptation (as tested by us in chapter 5) is independent to direction (forward or backward walking) and leg (right versus left) [80]. The main circuit underlying this form of motor adaptation is the cerebellum, and these results therefore suggest the capacity of a cerebellar functional differentiation to motor task demands. It would be interesting to investigate patients with the rare phenomenon of leg dystonia specific to forward walking, to see if this translates in *direction specific impairments* of walking adaptation.

III. In this thesis, we discussed the recent animal studies that have shown previously unknown pathways of communication between the basal ganglia and the cerebellum and their possible relevance in motor functioning [16-18]. This higlights that although the principal anatomy of the cerebellum might be defined, there is still knowledge to be gained regarding full understanding of the cerebellar architecture and connectome. Resting state functional MRI, together with MR diffusion tractography is currently used to achieve this. For example, subregions of the cerebellar cortex have been defined based on their functional connectivity with the cerebral cortex and cerebellar white matter tracts have been estimated using probabilistic tractography [81, 82].

The somatotopy of the cerebellum is also still under investigation, but there appears to be a cortical cerebellar topography. This topography is complex as body segments can be represented in different regions, different body parts can be represented in a single region, and as in the motor cortex, the cortical size of a body part embodies its functional importance instead of physical size [83-87].

Recent animal experiments showed that the extent of cerebellar dysfunction was found to determine the topographical extent of abnormal movements in a dystonia rodent model; this might explain why various forms of idiopathic dystonia are limited to different body parts [26].

Structural cerebellar lesions can cause acquired dystonia. In the future, one could possibly explore if the affected body part(s) somehow correspondences with the currently proposed

cerebellar somatotopy of the lesion site in these patients; particularly now new imaging methods with higher spatial discriminatory abilities are arising.

There have been various neurophysiological, behavourial and imaging studies investigating altered representations of affected body parts in the somatosensory cortex and basal ganglia of patients with idiopathic focal dystonia [88-91]. One could design functional imaging tasks to investigate if there is also a disturbed somatotopic cerebellar arrangement, possibly with new imaging methods offering the ability to image at a higher spatial resolution.

IV. Sensory dysfunction is often seen in idiopathic dystonia studies. Abnormalities in tactile discrimination tasks, proprioceptive abnormalities and impairments in sensory-motor integration have all been observed [92]. The extensive network behind sensory-motor integration includes not only frontal and parietal cortical areas, but also subcortical structures like the basal ganglia and cerebellum.

The basal ganglia may not directly receive sensory information but could serve as a gate-keeper for sensory input at various levels of the central nervous system. The cerebellum, however, receives sensory information directly, and could use this information for online correction of movement by error signals that reflect the difference between expected and observed sensory cerebellar input [66]. Mismatch negativity (MMN) is a negative component of the event related potential (ERP) that is calculated by subtracting the ERP from a standard repeated stimulus from that reproduced by a rare "oddball" stimulus in EEG (electroencephalography) studies. Aberrant somatosensory MMN has recently been observed together with normal auditory MMN in idiopathic dystonia (results not yet published).

Bhanpuri et al. assessed proprioception during passive arm movements, active arm movements with simple dynamics, and arm movements in a force field with unpredictable dynamics between healthy controls and patients with cerebellar disease [93]. They observed that during passive proprioception cerebellar patients and controls have the same precision. During active moment, proprioception improves only in controls, whereas in a force field with unpredictable dynamic their precision worsens to passive levels.

These results suggest that the mechanism to improve active versus passive proprioception is cerebellum-dependent and could indicate that cerebellar function enhances proprioception by predicting movement outcomes based on internal models of arm dynamics. The capacity to improve active versus passive proprioception could be studied in idiopathic focal dystonia.

V Cerebellar online motor correction signals during motor tasks could be produced via (in) direct modulation of M1 activity via the thalamus or via modulation of secondary motor areas [94-96]. There is however ongoing research to the efferent organization of the cerebellum and whether it has the capacity to subcortically elicit body movements. Mottolese et al. (2013) electrically stimulated the posterior cerebellum of twenty patients undergoing surgery [87]. The latency of the ipsilateral evoked body movements combined with results of previous animal studies suggest that the cerebellum might directly send corrective commands

via brainstem structures, perhaps via the reticulo-spinal tract [97]. These direct corrective commands could ensure rapid online motor corrections.

Better physiological knowledge regarding cerebellar anatomy and functioning would benefit studies of movement disorders, including dystonia.

This issue should be further explored, possibly by studies using TMS. One could perhaps test if and by which latency single TMS pulses over the cerebellum disrupt online movement correction and compare this with hemispheric cortical TMS pulses.

VI. The cerebellum has been hypothesized to play a role in coordinating the timing of different muscle groups in order to produce fluent body movement, but is thought to also mediate time perception [98]. Studies investigating timing in idiopathic focal dystonia have generated contrasting results.

In chapter 5, I found that patients with writer's cramp were impaired in temporal parameters of walking adaptation (double support time, phasing). However, the timing of conditioned responses during eyeblink classical conditioning was not different between patients and controls in chapter 4. As the table in the discussion section shows, no timing differences were observed in other cerebellar tasks between healthy controls and patients with idiopathic focal dystonia. Van der Steen et al. (2014) also recently reported normal performance on a sensorimotor synchronization task in patients with musician's dystonia [99]. But studies investigating the temporal variability of timed movements and/or time discriminating tasks in dystonia appear limited. Avanzino et al. (2013) found that the accuracy of predicting the end of a visually perceived human body movement is lower in patients with writer's cramp; these deficits were selective to human body movement (and not due to general timing impairments) [100]. This suggests that the cognitive processing of temporal components of body movement is less effective in patients than in healthy controls. More studies focused on this subject could help clarify the presence and extent of (motor) timing deficits in idiopathic dystonia and explore a possible role for the cerebellum in these abnormalities.

VII. If aberrant cerebellar functioning is (in part) responsible for sensory and cognitive aspects related to movement processing and planning in dystonia, there could also be a role for the cerebellum in the non-motor components of idiopathic dystonia. There appear to be abnormalities in neuropsychiatric, cognitive, and sleep domains in idiopathic dystonia patients [101]. However, there are discrepancies between studies that have explored the extent and domains of cognitive impairments in dystonia [102]. It is now clear that the cerebellum exerts a significant influence over non-motor cortical areas including regions of the prefrontal and posterior parietal cortex. The output originates from the dentate nucleus, which is divided into separate motor and non-motor domains. Neuroimaging and neurophysiological data support the effect of the cerebellum on non-motor cortical areas [96].

Dysfunction in fronto-striatal circuitry has been suggested as a neurobiological explanation for the higher incidence of neuropsychiatric features in idiopathic dystonia [101]. Further studies may clarify whether there is also a possible contribution of the cerebellum to that abnormal circuitry.

VIII. The studies in this thesis, and the above-suggested topics for further research, focus on the role of the cerebellum in idiopathic focal dystonia. However, I will finish this section with a short note on other motor abnormalities in dystonia and dystonia in other movement disorders, because I believe that the cerebellum could possibly play a role in their occurrence as well.

One of the motor abnormalities that can accompany dystonia is dystonic tremor. As Helmich et al. (2011) reported, resting tremor in Parkinson's disease may result from a pathological interaction between the basal ganglia and the cerebello-thalamic circuit [103]. A faulty interaction of these two subcortical circuits may also be responsible for the generation of dystonic tremor and future studies might clarify this. Our group will now start investigating the tremor circuit in dystonic versus essential tremor patients, combining functional MRI, MR-spectroscopy, and transcranial modulation of motor cortex and cerebellum.

Antelmi et al. recently reported a difference in eyeblink classical conditioning capacity between dystonic patients with and without tremor, supporting the hypothesis of a cerebellar role in the genesis of dystonic tremor [59].

Dystonia affecting the leg also frequently occurs in patients with known basal ganglia disorders such as Parkinson 's disease. Studies investigating dystonia co-occurring with other movement disorders, possibly with a control group of patients without dystonia, might help us to start understanding what triggers the manifestation of dystonid in these patients. For example, normal eyeblink classical conditioning (a neurophysiological indicator of cerebellar dysfunction) has been found in patients with Parkinson's disease, but is eyeblink classical conditioning perhaps abnormal in patients with Parkinson's sisease and leg dystonia [104]?

Conclusion

The search for the role of the cerebellum in the pathophysiology of dystonia has only recently begun. My studies have reinforced the concept of aberrant cerebellar functioning in idiopathic focal dystonia. However, the exact role of the cerebellum in the pathophysiology of idiopathic focal dystonia remains uncertain.

My studies show that the role of the cerebellum in dystonia appears to be more complicated than simply a loss or gain of cerebellar function. The role of the cerebellum may not be confined to one of the above theories, it might be more dynamic, and for example be partly pathological and partly compensatory engaged, depending on the executed (motor) task.

The role of the cerebellum might also differ for various types of dystonia. Dystonia appears to be a motor symptom that can reflect different pathophysiological states triggered by a variety of insults. Although there is probably a common pathophysiological pathway, divergence

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of pathways must exist to account for different forms of (idiopathic) dystonia. I observed different abnormalities, in degree and area, of cerebellar malfunctioning in various forms of idiopathic focal dystonia. This argues against uniform cerebellar pathology as a primary driver of dystonia.

In this thesis, I have outlined the current evidence that explores a possible role for the cerebellum in idiopathic focal dystonia. I provided additional new insights, but am today unable to draw final conclusions as more studies are warranted to further dissect the role of the cerebellum in this movement disorder.

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Chapter 7.1

7.2

Nederlandse samenvatting

Dystonie

Dystonie is een bewegingsstoornis die voor het eerst beschreven werd in 1911 door Oppenheim. De geschatte prevalentie is 15-30/100.000[1]. Dystonie wordt gekenmerkt door onwillekeurige aanspanning van spieren resulterend in (vaak repeterende) abnormale bewegingen en/of een gestoorde lichaamshouding. Dystonie wordt vaak veroorzaakt of neemt toe bij bepaalde handelingen. Een voorbeeld hiervan is taakspecifieke dystonie bij schrijven of bij het spelen van een muziekinstrument[2].

Er bestaan verschillende vormen van dystonie. Deze vormen worden ingedeeld op basis van klinische kenmerken en oorzaak. Klinische kenmerken zijn bijvoorbeeld: leeftijd bij start symptomen, getroffen lichaamsdelen (bijvoorbeeld een bepaald lichaamsdeel/focaal of meerdere lichaamsdelen/gegeneraliseerd), en bijkomende verschijnselen. Dystonie kan namelijk samengaan met andere bewegingsstoornissen en met andere neurologische of systemische verschijnselen. Wat betreft de oorzaak, kan dystonie erfelijk zijn (op basis van een fout in de genetische code), verworven (bijvoorbeeld door gebruik van medicatie of na een herseninfarct) of idiopathisch. Het laatste impliceert een onbekende of niet bewezen erfelijke dan wel verworven oorzaak[2].

Cervicale dystonie, schrijfkramp en blepharospasme zijn de meest voorkomende vormen van idiopathische focale dystonie die (meestal) op laat-volwassen leeftijd ontstaan. Cervicale dystonie wordt gekenmerkt door abnormale hoofd, nek en schouderbewegingen. Bij blepharospasme zorgt de onwillekeurige aanspanning van spieren rondom het oog voor onwillekeurige sluiting hiervan. Bij schrijfkramp induceert schrijven dystonie in de arm en hand. In dit proefschrift ligt de focus op deze drie vormen van focale idiopathische dystonie.

Dystonie is een invaliderende aandoening die gepaard kan gaan met pijn en depressie. Er zijn voor de meeste vormen van dystonie alleen symptomatische behandelingen omdat de mechanismen die leiden tot deze bewegingsstoornis voor een groot deel onduidelijk zijn. De basale ganglia (basale kernen) en het cerebellum (kleine hersenen) zijn beiden belangrijke hersengebieden voor normale motoriek.

Van oudsher werden de basale ganglia verantwoordelijk geacht voor het ontstaan van dystonie. Maar nieuwe studies en bijkomende inzichten hebben ervoor gezorgd dat er nu veel aandacht is voor de rol van het cerebellum[3].

Het doel van dit proefschrift was de rol van het cerebellum in het ontstaan van idiopathische focale dystonie verder te onderzoeken.

Er werden hiervoor verschillende methodes gebruikt, welke ik kort zal toelichten. Bij *transcraniële magnetische stimulatie (TMS)* wordt er door een spoel een korte magneetpuls gegeven welke een kleine stroom opwekt die neuronen (zenuwcellen) kan activeren. Als deze over de motorische cortex (de hersenschors verantwoordelijk voor de uitvoering van beweging) wordt gegeven, resulteert dit in spieractiviteit welke d.m.v. speciale huidelectrodes

boven deze spieren kan worden gemeten. Reeksen van pulsen (repetitieve TMS) over een bepaald gebied kunnen ervoor zorgen dat de verbindingen tussen neuronen tijdelijk sterker of minder sterk worden, waardoor het hersengebied meer of minder actief zal zijn[4].

Oogknipper conditionering is een vorm van associatief leren waarbij gepaarde presentatie van een geluid gevolgd door een elektrische stimulus of luchtpufje bij het oog ervoor zorgt dat het oog uiteindelijk reflexmatig wordt gesloten bij het horen van het geluid alleen. Het wordt beschouwd als een vorm van impliciet oftewel onbewust leren, waar het cerebellum een hele belangrijke rol bij speelt[5-7].

Vicon is een systeem waarbij menselijke bewegingen driedimensionaal kunnen worden opgenomen. Dit gebeurt door reflectieve markers op het lichaam aan te brengen welke dan door speciale camera's vanuit verschillende richtingen worden gefilmd. *Functionele MRI (fMRI)* is een speciale MRI techniek. Bij dit type onderzoek wordt vaak gevraagd aan deelnemers om een taak uit te voeren in de MRI-scan. Er wordt op deze wijze een 3D afbeelding van de hersenen gemaakt. Hierbij wordt activiteit in hersengebieden op een computer in beeld gebracht doordat verhoging van activiteit gepaard gaat met een toename van doorbloeding welke met fMRI wordt vastgelegd[8].

Overzicht proefschrift

Hoofdstuk 2 is een overzicht van de studies, beschikbaar bij aanvang van dit promotieonderzoek, die een rol voor het cerebellum in het ontstaan van dystonie ondersteunen.

In **hoofdstuk 3.1** onderzocht ik d.m.v. TMS de prikkelbaarheid van de motor cortex bij patiënten met een syndroom van dystonie en cerebellaire ataxie (DYTCA)[9, 10]. Cerebellaire ataxie is een coördinatiestoornis berustend op een gestoorde werking van het cerebellum. Ik vond dat de prikkelbaarheid van de motor cortex in deze patiëntengroep meer lijkt op die van patiënten met aandoeningen van het cerebellum dan van patiënten met idiopathische focale dystonie[11-16]. De prikkelbaarheid van de motor cortex is dus niet hetzelfde bij iedere vorm van dystonie en het cerebellum lijkt de prikkelbaarheid van de motor cortex (indirect) te beïnvloeden. Dit onderzocht ik verder in **hoofdstuk 3.2**, waarin ik met TMS onderzocht of de invloed van het cerebellum op het verlagen van de prikkelbaarheid van de motor cortex bij het starten van een beweging selectief is voor de spieren die betrokken zijn bij deze beweging[17, 18]. Bij gezonde mensen is namelijk het gedeelte van de motor cortex verantwoordelijk voor de spieren die de beweging uitvoeren op dat moment actief (toegenomen prikkelbaar) en de gedeeltes die bij omliggende spieren horen minder actief (verminderd prikkelbaar), zodat de beweging nauwkeurig kan worden uitgevoerd. Dit fenomeen heet in het Engels 'surround inhibition'. Bij patiënten met dystonie is dit gestoord, waardoor ook het gedeelte van de motor cortex verantwoordelijk voor de omliggende spieren actief is [19]. Ik vond niet dat het cerebellum direct verantwoordelijk is voor 'surround inhibition' bij het starten van een beweging, maar wel dat de cerebellaire remming van de prikkelbaarheid van de motor cortex voor alle spieren bij de start van een beweging afneemt, hierdoor kunnen andere

hersengebieden wellicht hun invloed op de prikkelbaarheid van de motor cortex beter uitoefenen.

In **hoofdstuk 4** onderzocht ik oogknipper conditionering, een vorm van associatief leren waarvoor het cerebellum het belangrijkste verantwoordelijke onderliggende hersengebied is. Oogknipper conditionering is afgenomen bij patiënten met idiopathische cervicale dystonie, maar met extra sessies kunnen deze patiënten oogknipper conditionering wel verbeteren[20]. Door reeksen van TMS pulsen (repetitieve TMS) over de schors van het cerebellum te geven wordt dit hersengebied tijdelijk minder actief. We zagen hierdoor een afname van oogknipper conditionering in gezonde deelnemers maar een toename van oogknipper conditionering in patiënten met idiopathische focale dystonie. Het functioneren van het cerebellum is dus te beïnvloeden door training, maar ook door repetitieve TMS in deze patiëntengroep.

De reactie van het cerebellum bij patiënten met idiopathische cervicale dystonie op repetitieve TMS is dus anders dan bij gezonde mensen. Er is meer onderzoek nodig om te verklaren waarom het cerebellum overactief is bij patiënten met idiopathische focale dystonie.

Bij patiënten met gefixeerde dystonie was oogknipper conditionering normaal. Afgenomen oogknipper conditionering lijkt dus een selectief kenmerk te zijn voor patiënten met idiopathische focale dystonie.

In **hoofdstuk 5** onderzocht ik met Vicon beweging op een lopende band waarbij de loopsnelheden voor het linker en rechter been verschillend waren. Het looppatroon moet hierop worden aangepast. Deze vorm van motor adaptatie wordt voor een groot deel gereguleerd door het cerebellum[21]. Dit bleek minder goed te verlopen bij patiënten met focale hand dystonie en patiënten met blepharospasme. De problemen met het leren van motorische taken zijn dus uitgebreider dan alleen oogknipper conditionering. De resultaten van patiënten met cervicale dystonie waren niet significant verschillend van de controle groep. De (abnormale) werking van het cerebellum lijkt dus anders voor diverse vormen van idiopathische focale dystonie.

Hoofdstuk 6 was een fMRI studie waarin patiënten met cervicale dystonie werd gevraagd twee verschillende leertaken uit te voeren. In een taak werd een nieuwe volgorde van hand bewegingen aangeleerd. In de andere taak werd geleerd plaatjes te koppelen aan specifieke handbewegingen. Voor de eerste taak weten we dat het cerebellum een belangrijke rol speelt in deze specifieke vorm van bewegingen aanleren; voor de tweede taak zijn dat de basale ganglia[22-27]. Bij het leren van de volgorde bewegingen was het cerebellum bij patiënten extra actief en de mate van activiteit correleerde positief met het uitvoeren van de taak. Dus bij meer activiteit werd de taak beter uitgevoerd. Bij het leren plaatjes aan handbewegingen te koppelen waren de basale ganglia bij patiënten verminderd actief. Mogelijk compenseert het cerebellum dus voor basale ganglia dysfunctie bij deze vorm van idiopathische dystonie, maar er werd geen toegenomen cerebellaire activiteit simultaan met afgenomen basale ganglia activiteit geobserveerd in één taak. De (dys)functie van het cerebellum en de basale ganglia bij patiënten met idiopathische focale dystonie lijkt dus gebonden aan de context waarin een beweging wordt uitgevoerd.

Conclusie

Onderzoek naar de rol van het cerebellum in het ontstaan van dystonie is pas recent gestart. De studies van dit proefschrift ondersteunen het abnormaal cerebellair functioneren in dystonie. De exacte rol van het cerebellum en de interactie van het cerebellum met de basale ganglia zijn echter tot op heden onbekend.

Het cerebellum en de basale ganglia spelen allebei een eigen en belangrijke rol in het coördineren van bewegingen. Dystonie kan worden verworven door letsel van beide structuren[28, 29]. Allebei deze hersengebieden lijken dus het vermogen te hebben om (in)direct dystonie te veroorzaken. Door nieuwe anatomische studies weten we nu dat er directe communicatie tussen deze twee gebieden mogelijk is[30]. Chen et al. bewees recent het cerebellair moduleren van basale ganglia functie en het belang daarvan in normale motoriek[31]. Deze studie en verschillende dierstudies opperen dat het cerebellum primair verantwoordelijk is voor het ontstaan van dystonie[32-35]. Een gestoorde werking van het cerebellum kan ook mogelijk secundair leiden tot basale ganglia dysfunctie en zo dystonie veroorzaken. Het is echter onduidelijk in hoeverre de resultaten van dierstudies kunnen worden geëxtrapoleerd; de dierstudies zijn vaak gebaseerd op erfelijke vormen van dystonie of op dystonie in combinatie met andere neurologische symptomen.

Een andere theorie is dat het cerebellum compenseert voor dysfunctie van andere hersengebieden, bijvoorbeeld de basale ganglia. Mijn fMRI-studie waarin handbewegingen op verschillende manieren werden geleerd lijkt dit te ondersteunen, samen met andere recente studies in idiopathische dystonie[36, 37].

Gestoorde oogknipper conditionering en een veranderde invloed van het cerebellum op de prikkelbaarheid van de motor cortex in idiopathische focale dystonie kunnen beide theorieën ondersteunen, want compensatoire of secundaire aanpassingen kunnen ook mogelijk normaal cerebellair functioneren verstoren.

De rol van het cerebellum is mogelijk niet beperkt tot één van deze theorieën. Mogelijk is de werking gedeeltelijk pathologisch en/of gedeeltelijk compensatoir. In mijn fMRI-studie was de (dys)functie van deze hersengebieden gebonden aan de context waarin een beweging werd uitgevoerd. De rol van het cerebellum kan ook verschillend zijn voor diverse vormen van dystonie. Dit zag ik in mijn loopbandstudie bij verschillende vormen van idiopathische focale dystonie.

Er is meer onderzoek nodig om de rol van het cerebellum in deze bewegingsstoornis verder te verhelderen.

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Chapter 7.2

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Appendices

A1 List of abbreviations

A-period	adaptation period
ABC-NL	Dutch version of the activities-specific balance confidence scale
ADM	abductor digiti minimi
AMT	active motor threshold
ANOVA	analysis of variance
BG	basal ganglia
BOLD	blood-oxygen-level dependent
BSP	blepharospasm
CBI	cerebellar brain inhibition
CD	cervical dystonia
CER	cerebellum
CR	conditioned eyeblink response
CRPS	complex regional pain syndrome
CS	conditioning stimulus (chapter 3.2) / conditioned stimulus (chapter 4)
CSP	cortical silent period
DCS	direct current stimulation
DS	double support
DTI	diffusion tensor imaging
DYT	patients with primary focal or segmental dystonia
DYTCA	syndrome of dystonia and cerebellar ataxia
DYT*t-plus*	patients with primary focal or segmental dystonia that had arm/hand involvement
EBCC	eyeblink classical conditioning
EMG	electromyography
ERP	event related potential
FA	fractional anisotropy
FDI	first dorsal interosseus
F-period	fast walking period
GABA	gamma-aminobutyric acid
HRF	hemodynamic response function
ICF	intracortical facilitation
ISI	interstimulus intervals
I-waves	indirect waves
LTD	long-term depression
LTP	long-term potentiation
M1	primary motor cortex
MEP	motor evoked potential
MMN	mismatch negativity

MNI	montreal neurological institute
NMRP	normal motor-related activation pattern
OO	orbicularis oculi
fMRI	functional magnetic resonance imaging
PAS	paired associative stimulation
PC	purkinje cells
PET	positron emission tomography
PMC	premotor cortex
P-period	post adaptation period
RMT	resting motor threshold
ROI	region of interest
S1-period	first slow walking period
S2-period	second slow walking period
SD	standard deviation
SEM	standard error of the mean
SI	stimulus intensity (chapter 3.1) / surround inhibition (chapter 3.2)
SICI	short interval intracortical inhibition
SMA	supplementary motor area
SPM	statistic parametric mapping
TS	test stimulus
TWSTRS	Toronto western spasmodic torticollis scale
TBS	theta burst stimulation
cTBS	continuous theta burst stimulation
iTBS	intermittent theta burst stimulation
rTMS	repetitive transcranial magnetic stimulation
TMS	transcranial magnetic stimulation
US	unconditioned stimulus
WC	writer's cramp

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A3   About the author

Britt Sofie Hoffland was born on the 11th of February 1987 in Amsterdam, the Netherlands. She is the middle child of Monique Kenter and George Hoffland. She has an older brother named Stijn and a younger sister named Juulke. She graduated from secondary school at the Bouwens van der Boijecollege in Panningen in 2005. Britt obtained her medical degree in 2014 at the Radboud University Nijmegen. As part of her medical studies, Bart van de Warrenburg gave her the opportunity to complete a research internship at the Sobell Department of Motor Neuroscience and Movement Disorders at Queensquare London, introducing her to idiopathic focal dystonia. This research internship was partly funded with a student grant from the Prinses Beatrix Fonds (now the Prinses Beatrix Spierfonds). She temporarily paused her medical studies after this internship to start a PhD project on the role of the cerebellum in the pathophysiology of idiopathic focal dystonia, resulting in this thesis. The work of this thesis was, among others, presented at the Movement Disorders Society International Congress in Buenos Aires (2010) and the International Dystonia Symposium in Barcelona (2011) where it was rewarded with the prize of best poster abstract.
Britt started working as neurology resident at the Neurology department of the Radboudumc in 2015. In her free time she enjoys playing field hockey, piano, reading and drawing.

Britt lives together in Nijmegen with Niek and their daughter Noor (30-10-2017).

A4   List of publications

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Neuromuscular disorders of movement

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A6   Donders Graduate School for Cognitive Neuroscience

For a successful research Institute, it is vital to train the next generation of young scientists. To achieve this goal, the Donders Institute for Brain, Cognition and Behaviour established the Donders Graduate School for Cognitive Neuroscience (DGCN), which was officially recognised as a national graduate school in 2009. The Graduate School covers training at both Master's and PhD level and provides an excellent educational context fully aligned with the research programme of the Donders Institute.

The school successfully attracts highly talented national and international students in biology, physics, psycholinguistics, psychology, behavioral science, medicine and related disciplines. Selective admission and assessment centers guarantee the enrolment of the best and most motivated students.

The DGCN tracks the career of PhD graduates carefully. More than 50% of PhD alumni show a continuation in academia with postdoc positions at top institutes worldwide, e.g. Stanford University, University of Oxford, University of Cambridge, UCL London, MPI Leipzig, Hanyang University in South Korea, NTNU Norway, University of Illinois, North Western University, Northeastern University in Boston, ETH Zürich, University of Vienna etc.

Positions outside academia spread among the following sectors:
- specialists in a medical environment, mainly in genetics, geriatrics, psychiatry and neurology,
- specialists in a psychological environment, e.g. as specialist in neuropsychology, psychological diagnostics or therapy,
- higher education as coordinators or lecturers.

A smaller percentage enters business as research consultants, analysts or head of research and development. Fewer graduates stay in a research environment as lab coordinators, technical support or policy advisors. Upcoming possibilities are positions in the IT sector and management position in pharmaceutical industry. In general, the PhDs graduates almost invariably continue with high-quality positions that play an important role in our knowledge economy.
For more information on the DGCN as well as past and upcoming defenses please visit:

http://www.ru.nl/donders/graduate-school/phd/