The role of the cerebellum in the pathophysiology of dystonia

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PhD thesis

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‘I, Anna Sadnicka, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis’

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Date:

Abstract

Research over the last decade has refined our understanding of the neuroanatomical substrates of dystonia. In addition to basal ganglia dysfunction a much wider sensorimotor network has been implicated and within this network the cerebellum is heralded as a core node. Much of the literature linking the cerebellum to dystonia consists of cases in which lesions of the cerebellum are linked to abnormal posture or indirect experimental associations (reviewed in chapter 1). Better defining the functional role of the cerebellum in the pathophysiology of dystonia could provide a scientific rational for future therapeutic advances, adding further weight to an early neurosurgical literature which advocates targeting the cerebellum and its outflow tracts.

Within this thesis I applied experimental techniques from which direct inferences about cerebellar function could be made, trying to better define *how* the cerebellum is functionally involved in the pathogenesis of isolated dystonia. Methodology can be divided into major themes (i) two studies exploring cerebellar modulation of dystonic neurophysiological hallmarks; impaired motor surround inhibition (chapter 2) and excessive plasticity (chapter 3) (ii) evaluation of eye-blink conditioning a form of cerebellar associative learning (chapter 4, chapter 8) (iii) exploring whether millisecond timing, a cerebellar encoded process, is at the root of abnormal temporal discrimination thresholds (chapter 5) and finally (iv) testing adaptation a kinematic cerebellar paradigm in cervical dystonia (chapter 6) and DYT1 dystonia (chapter 7).

Overall, my application of the 'purest' cerebellar paradigms did not provide a robust functional correlate to implicate specific cerebellar functions as a driver of dystonic pathophysiology. I present good evidence that fundamental computations such as adaptation and associative learning are intact in various groups of isolated dystonia. Thus isolated dystonia does not seem to selectively impair cerebellar functions (as currently defined). It is only with future research that we will be able to determine whether dystonia corrupts function(s) inherent to the dystonic network which includes the cerebellum or whether the cerebellar abnormalities observed experimentally are compensatory in nature.

Acknowledgments

I thank my supervisors Mark and John that have guided me so exceptionally and for welcoming me into this wonderful research family. I have had the privilege of working with many talented colleagues who taught me experimental technique, analysis and programming: Panagiotis Kassavetis, Isabel Pareés, Tabish Saifee, Maja Kojovic, Masashi Hamadi, Sanjay Manohar and Joe Galea. Thank you to the students or visiting neurologists that I supervised in the latter part of the PhD to collect some of the experimental data for the following projects: Corinna Daum – millisecond timing mechanisms in cervical dystonia, Bansi Patani - adaptation in cervical dystonia, Anna Stevenson - adaptation in DYT1 dystonia. I also thank the patients who gave their time to these projects - without their enthusiasm this work would not have been possible.

I dedicate this thesis to my parents Michael Sadnicki and Alison Sadnicka – a formidable duo that will always inspire me in all walks of life. To Lucia and Paula, born during this PhD - thank you for my parallel existence within a world of princesses and estrellitas. Finally to Diego, my husband – you've allowed me the highs and lows of research life, supported us financially but above all kept the poetry of life ticking. Soy nada sin vos y te amo.

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Abbreviations

a	decision boundary
ADM	abductor digiti minimi
AIC	Akaike Information Criterion
AMT	active motor threshold
APB	abductor pollicis brevis
BRR	blink recovery reflex cycle
CBI	cerebellar brain inhibition
CD	cervical dystonia
cDC	transcranial direct current stimulation of the cerebellum
CF	climbing fibre
COV	coefficient of variation
CR	conditioned response
CS	conditioning stimulus
CSP	cortical silent period
cTBS	continuous theta burst stimulation
DBS	deep brain stimulation
DTI	diffusion tensor imaging
DYTCA	dystonia with cerebellar atrophy
EBC	eye blink conditioning
EMG	electromyography
FDI	first dorsal interosseus

fMRI	functional magnetic resonance imaging
FP	false positive
GABA	γ-aminobutyric acid
GPi	globus pallidus internus
INC	interstitial nucleus of Cajal
LTD	long term depression
LTP	long term potentiation
MEP	motor evoked potential
MF	mossy fibre
MLI	molecular layer interneurons
mSI	motor surround inhibition
MSO	maximum stimulus intensity
mu	mean
nD	non Decision time
nMEP	normalised PAS response
PAS	paired associative stimulation (PAS)
PAS25	PAS with interstimulus interval of 25ms
PC	Purkinje cell
PET	positron emission tomography
PF	parallel fibre
RC	recruitment curve
rRC	linear regression of RC
rmANOVA	repeated measures analysis of variance

RMT	resting motor threshold
SCA3	spinocerebellar ataxia type 3
SEM	standard error of the mean
sigma	standard deviation
SI	stimulus intensity
TDT	temporal discrimination threshold
TMS	transcranial magnetic stimulation
TWSTRS	Toronto Western Spasmodic Torticollis Rating Scale
UR	unconditioned response
US	unconditioned stimulus
v	drift rate
VAS	visual analogue scale
WCRS	Writer’s Cramp Rating Scale
WD	Writing dystonia

1 Introduction

1.1 Dystonia as a network disorder

The dystonias are a heterogenous group of hyperkinetic movement disorders. They are characterised by involuntary sustained muscle contractions which lead to twisting and repetitive movements or abnormal postures of the affected body part[1]. Dystonia is the third most common movement disorder worldwide and affects approximately 70,000 people in the UK[2]. Classically dystonia was considered a disorder of basal ganglia dysfunction[3]. However more recently research in animals and humans has pointed to abnormalities of multiple brain regions[4]. Within this wider sensorimotor network the cerebellum is thought to be a key node[5].

The role of the cerebellum in the pathophysiology of dystonia is a growing field of interest (see Figure 1-1). However despite much attention to this topic, a major level of evidence within this story was missing: how could the cerebellum *functionally* contribute to dystonia pathophysiology. Can we provide mechanistic evidence that starts to unravel how cerebellar function is potentially disrupted in dystonia?

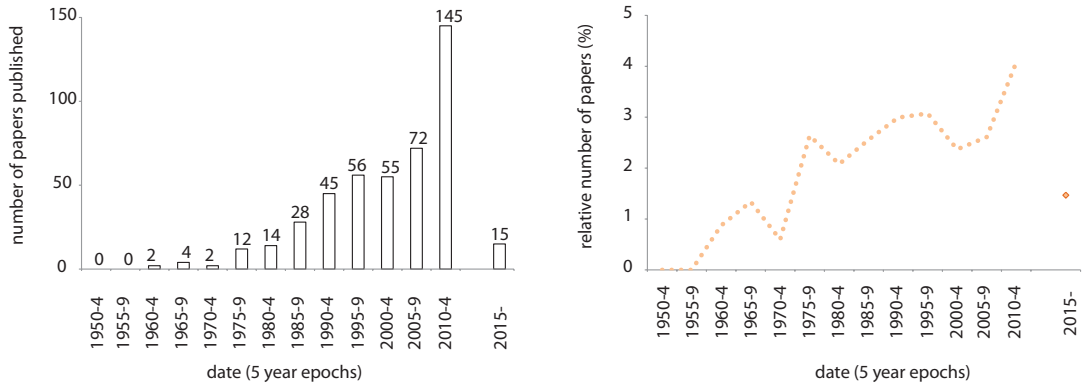


Figure 1-1 Publications on the cerebellum and dystonia

The bar plot shows the absolute number of papers indexed in pubmed using the search strategy "dystonia[all] AND cerebellum[all]" over the 5 year epochs indicated on the x-axis. The proportion of studies in relation to the total number of papers published on dystonia is also increasing (absolute number divided by total number of papers indexed in pubmed using the search strategy "dystonia[all]")

Exploring the functional role of the cerebellum in dystonia is the topic of this thesis. Within this introductory chapter I first explore the anatomy and function of the cerebellum in health. I then summarise evidence that links the cerebellum to dystonia. Drawing tentative conclusions I then define the hypothesis addressed by each of the subsequent experimental chapters. Gaining further understanding of the neuroanatomy of dystonia is important as it will guide more precise studies of physiology and aid the design of both medical and surgical treatment strategies.

1.2 The cerebellum in health

1.2.1 Anatomy

The basic internal organisation of the cerebellum is a highly convoluted cortex overlying a dense core of white matter. The cerebellar cortex contains a relatively small number of different cell types that are interconnected in a highly stereotyped way. The main anatomical layout of the cerebellum is shown in Figure 1-2. *Mossy fibers* (MF) provide the main input source to the cerebellum conveying information from multiple sources. These synapse on *granule cells* which branch into *parallel fibers* (PF) to relay information via excitatory synapses with *Purkinje cells* (PC). PCs have flat and elaborate dendritic trees that branch orthogonally to the direction of the PFs with each PC receiving input from over 150,000 granule cells. *Climbing fibers* (CF) which arise solely from the inferior olive provide additional sparser input as each PC receives input from a single CF (each CF branches to innervate ~ 10 PC). PC fire two types of spike. Simple spikes are normal action potentials and thought to be modulated by the many PF that contact the PC dendritic tree. By contrast, complex spikes are unique to PC and these only fire when the CF fires (the CF-PC synapse is one of the most powerful synaptic junctions of the central nervous system). The deep cerebellar nuclei are the only output of the cerebellum and are inhibited by activity in PC. Pauses in the firing of specific sets of PC therefore releases inhibition of cells within the deep cerebellar nuclei. One interpretation of this homogeneity is that a single computational function may characterize the role of the cerebellum across motor and non-motor domains. What constitutes this “universal cerebellar transform” however is a much-debated matter[6,7].

At the macroscopic level, in the anterior-posterior direction, a series of horizontally running fissures divide the cerebellum into a set of lobules (Figure 1-2) and distinctive functional roles are discernable within this lobular structure. Lobules I-V form the anterior cerebellum, and are primarily concerned with motor control. Lobules IV and V have reciprocal connections with primary motor cortex and have a reliable somatotopic organization[8,9]. Upper-limb representations are strongly lateralized with hand movements activating the ipsilateral anterior lobe almost exclusively[10]. Complex limb movements preferentially activate lobule VI with bilateral activation for unilateral hand movements acting with secondary motor

areas such as the premotor and supplementary motor areas[11]. Language tasks, including verb generation, also activate right lobule VI (and Crus I of lobule VII, see below), even when motor output-related activity is controlled for[12]. The vermis of lobules VI and VII controls eye movements and is known as the oculomotor vermis[13]. Lobule VII is the largest lobule of the human cerebellum and accounts for roughly half the cerebellar gray matter volume. Functionally, various language, working memory and executive function tasks activate lobule VII[14]. Lobules VIIIa and VIIIb are part of a second motor cortical–cerebellar loop[14]. As with the anterior motor representation, these lobules have a convergent representation of movement and sensory information from the whole body. While there is discernable somatotopy in these regions, it is weaker than that in the anterior lobe. The hemispheres of lobule IX has been linked autonomic function such as cardiovascular control and lobule X which incorporates the flocculus and nodulus, is concerned with vestibular function and eye movement control.

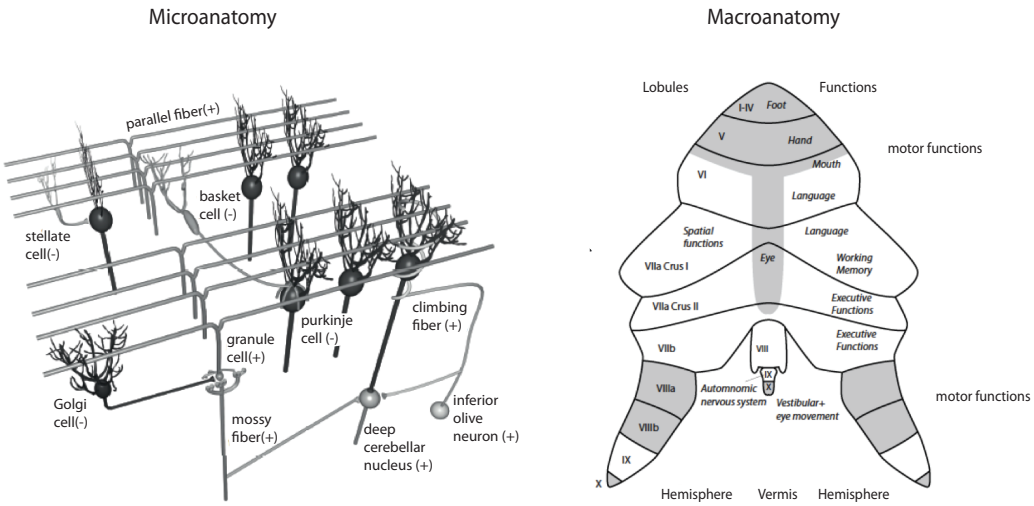


Figure 1-2 Cerebellar anatomy

Microanatomy: major cell types and their connections are displayed and the sign indicates whether the cell gives rise to excitatory (+) or inhibitory (-) connections. Macroanatomy: schematic representation of the major anatomical subdivisions and lobules of the cerebellum. A superior view of an "unrolled" cerebellum (adapted from Diedrichsen and Bastian[15]).

Another important anatomical feature in relation to the topic of this thesis is the reciprocal communication between the cerebellum and the basal ganglia. In the past, the cerebellum and the basal ganglia were thought to modulate cortical regions via distinct thalamic nuclei using separate parallel pathways[16]. However studies using viral tracers in primates revealed that there are substantial direct communications between the basal ganglia and the cerebellum: a disynaptic projection linking the dentate nucleus to the striatum[17], and a forward connection from the subthalamic nucleus of the basal ganglia to the cerebellar

cortex[18,19]. Such reciprocal communication between these two major subcortical structures strongly suggests that they directly modulate each other.

In human evolution the cerebellum has increased in absolute size relative to neocortical size and contains four times more neurons than the neocortex[20]. This cerebellar expansion is thought to have gifted advances in motor control but may also have provided advantages required for more complex human behaviour such as language[21].

1.2.2 Predictive controller

Once central theory about cerebellar function is that it may generate a predictive forward model for motor control[22]. Consider the scenario in Figure 1-3; a controller (in this case motor cortex) computes a motor command based on the goal and an estimate of the body's current state (i.e., position, velocity). Motor commands are sent to the muscles, causing them to contract and move the body. Feedback from the motor and sensory consequences of the movement could then close the feedback loop.

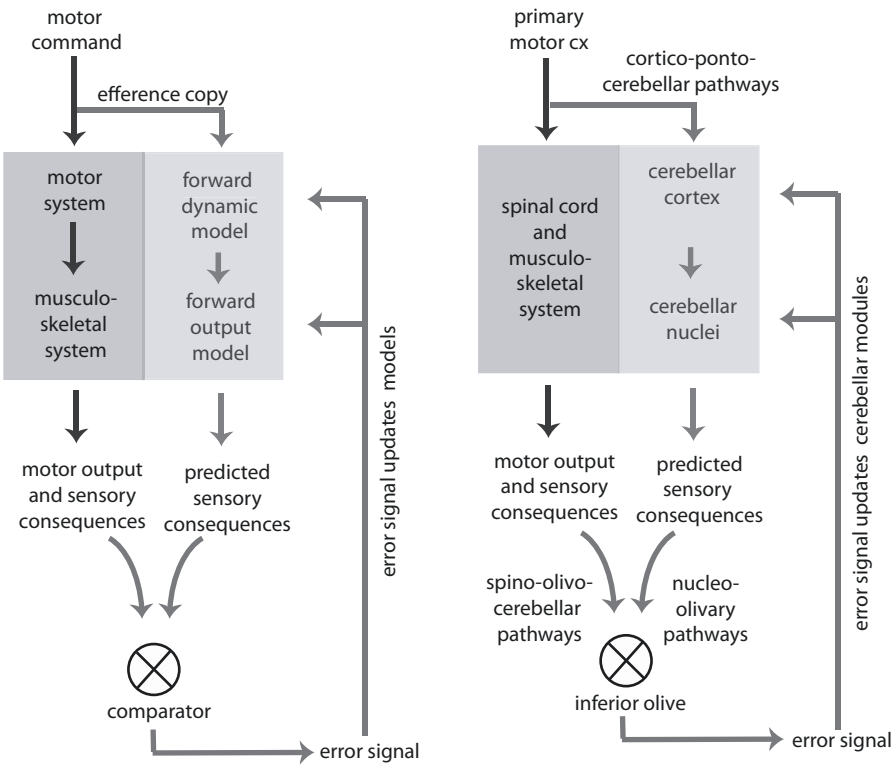


Figure 1-3 Theoretical and neural organisation of forward models.

On the left a theoretical model of how forward models are used is given. Motor commands directed to the musculoskeletal system are also copied to forward models which mimic these systems. Possible anatomical correlates of this theoretical control model is given on the right sided panel (adapted from Ramnani[23])

However movements are too fast to rely on such feedback (motor and sensory feedback delay can be in the order of 30-70ms) and such a loop would rapidly become unstable. It is thought that the solution is to employ a predictive device (the cerebellum) which uses a copy of the motor command (efference copy) to predict the future state of the limb. This prediction can then be integrated (accounting for sensory delays) with actual sensory feedback to produce an error signal to update future movement planning.

On a systems level, it remains unclear whether the cerebellum implements predictive forward models, or whether it merely adapts forward models stored elsewhere (see right sided panel of Figure 1-3). Evidence using transcranial direct current stimulation suggests that the cerebellum may produce short-term modifications of forward models, whereas the motor cortex may store longer-lasting motor memories[24].

1.2.3 Adaptation

An experimental paradigm which is thought to test predictive control is motor adaptation. In an example experiment the subject is required to adapt performance of a task (such as reaching to hit a target) after an environmental perturbation (such as distortion of visual feedback) introduces a movement error.

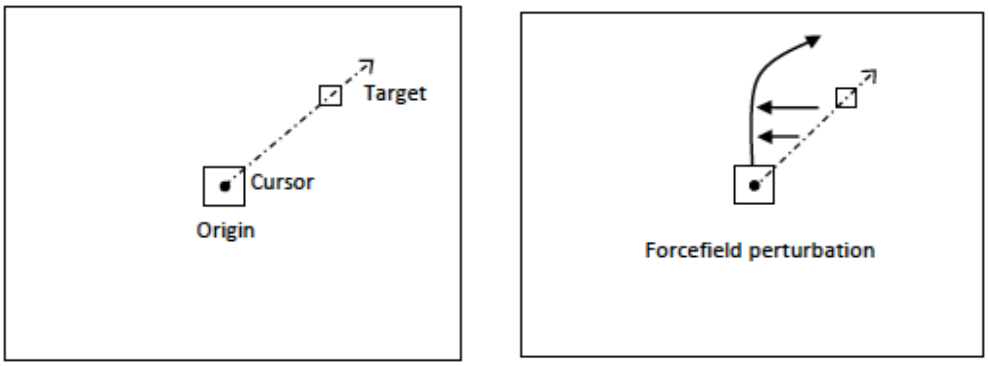


Figure 1-4 Example of an adaptation paradigm in humans

In a baseline task participants make reaching movements towards targets. During the adaption block a force is applied to the robotic arm during movement to induce error.

The sensory prediction error (how the actual sensory movement outcome differed from the predicted sensory movement outcome) is used to update subsequent motor performance[25]. As previously detailed it is thought that the cerebellum is crucial for the formation of forward models, which predict the sensory consequences of a motor command to drive adaptation[26,27]. Such mechanisms are likely to be continuously engaged correcting for small environment changes or performance fluctuations, keeping well-trained motor behaviors calibrated. Deficits across a range of adaptation tasks have been reported in patients with

cerebellar damage or degeneration. In force field adaptation tasks, a robotic device applies a systematic force to take the path away from its desired trajectory when people make point-to-point reaching movements (see Figure 1-4). Patients with cerebellar damage or degeneration are highly impaired in this learning task, evidenced by increased errors and reduced after effects[26,28]. They are also impaired in other adaptation experiments such as split-belt treadmill walking and reaching under novel visuo-motor transformations [27,29].

1.2.4 Eye blink conditioning

Pavlovian eye blink conditioning is another exceptionally well characterised cerebellar dependent experimental paradigm. The core experimental components are that a conditioning stimulus (CS) and an unconditioned stimulus (US) are paired together repetitively. The CS always occurs before the US. The US yields an unconditional blink response (UR). Over time the brain learns the pairing of the stimuli and a conditioned response (eye blink, CR) occurs before US. It can be tested across species and is commonly tested in mice, rabbits and more recently in the exploration of cerebellar diseases in humans (Figure 1-5).

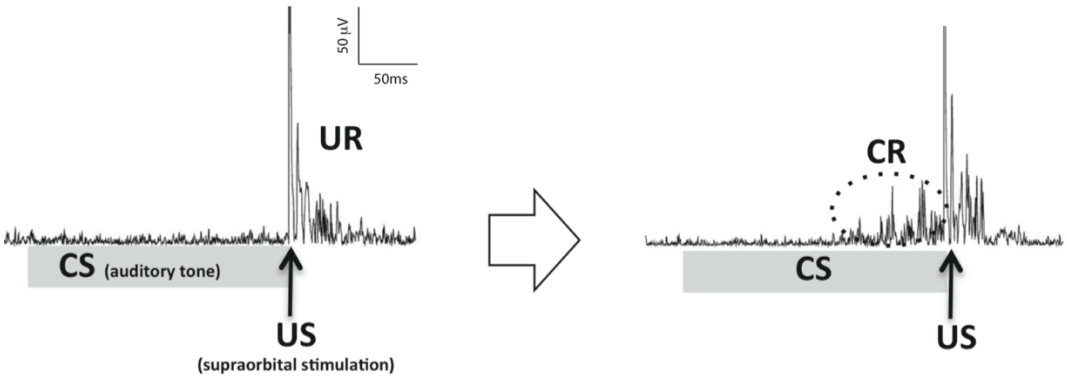


Figure 1-5 Eye blink conditioning in humans

Rectified electromyographic (EMG) recordings with time (ms) on the x-axis and voltage (µV) on the y-axis. The conditioning stimulus (CS) is a loud (70dB) 200Hz auditory tone lasting 400ms (shown by grey box). This occurs before an unconditioned stimulus (US) in which the supraorbital nerve is stimulated electrically (200µs, at five times the sensory threshold). Stimulation of the supraorbital nerve evokes an eye blink, the unconditioned response (UR), visible as increased EMG activity shortly after the stimulus is applied. After repeated pairings the conditioned response (CR) is visible before the supraorbital stimulation is delivered.

This paradigm is rather unique as components of the eye blink learning paradigm can be specifically mapped to the function of individual cells within the cerebellar circuitry. It can be shown that the magnitude of the conditioned eye blink responses correlates on a trial-by-trial manner to simple spike suppression of PCs. Complex spikes triggered by either the conditioning stimulus or unconditioned stimulus also predict the amount of simple spike

suppression/conditioned eyelid response. Molecular layer interneurons also appear to modulate their activity during the inter-stimulus interval and also predict the behavioural conditioning response (Figure 1-6).

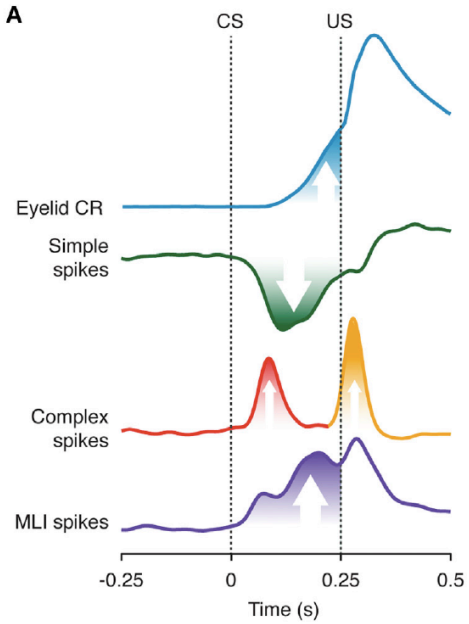


Figure 1-6 Cerebellar neuronal responses and conditioned response

This figure is taken from ten Brinke et al., in which cerebellar cortical electrophysiology and eyelid behaviour were simultaneously recorded in awake mice trained in an eye blink conditioning paradigm[30]. The traces are mean response of all cells in trained animals. At the top in blue is the mean eyelid conditioned response (CR). Simple spike (green), complex spike (red, yellow) and molecular layer interneurones spikes (MLI, purple) are plotted over time in seconds on the x-axis. The timing of the CS and US are shown by the dotted lines.

1.2.5 Millisecond timing

Eye blink conditioning can also be viewed as a timing task; the requirement to learn the association between two stimuli (US and CS) that are presented with a short inter-stimulus interval between them. The timing features of the task can also be further accentuated. For example if the animals are trained with 2 different inter-stimulus intervals between the US and CS on alternate trials then the response the animals learn will have two peaks, each corresponding to the different inter-stimulus intervals[31]. Interestingly the cerebellum may also have a role encoding such features of timing. Support for this is found across a range of task based on both motor and sensory timing experiments. Cerebellar activity is increased in the lateral cortex during experimental tasks such as interval discrimination[32] and correspondingly cerebellar patients have deficits in interval discrimination[33]. Computational cerebellar models also illustrate unique features of the cerebellar circuitry which make it a good candidate to encode timing in the millisecond range[34].

1.3 The cerebellum and dystonia

So what is the evidence that links the cerebellum to dystonia? In animal models modulating cerebellar function can cause or abolish dystonia. In humans, pathology of the cerebellum is associated with dystonia and an early neurosurgical literature supports the idea that modulation of cerebellar activity can attenuate the severity of dystonia. The expanding number of gene mutations identified for isolated dystonia sheds light on genetic neuroanatomy. There is also a growing experimental literature which associates cerebellar changes to isolated dystonia across a range of experimental modalities.

1.3.1 Animal models of dystonia

Animal models provide some of the most direct evidence to date that changes in cerebellar activity are important in dystonia pathophysiology. For example, tottering mice mutants exhibit paroxysmal dystonia due to a point mutation in a gene that codes for a calcium channel[35]. Clinically and electrophysiologically these episodes have characteristics similar to human dystonia[36]. Surgical removal of the cerebellum abolishes dystonic attacks in these mice[37] and elimination of dystonic movements following cerebellectomy has also been found in other murine models of dystonia[38,39]. Similarly, dystonia is abolished if the tottering mouse is bred with an additional genetic mutation that causes Purkinje cell degeneration[40]. In a pharmacological mouse model for dystonia, microinjection of low doses of kainic acid (which is neuroexcitatory) into the cerebellar vermis of mice generates dystonia of a severity proportional to kainite dose[41]. 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 neurotransmitters[37]. However after a string of publications firmly implicating cerebellar pathology in DYT1 dystonia a recent knock in mouse surprisingly revealed no motor deficits if the mutation is solely expressed in hindbrain structures[42]. These results may be partially explained by the fact that dystonia may require impairments in a motor network incorporating both the basal ganglia and the cerebellum as other models have shown how the basal ganglia may act as a filter for abnormal movement if intact[4].

1.3.2 Human cerebellar maladies and dystonia

It has long been recognised in both adult and paediatric neurology that posterior fossa tumours can present with cervical dystonia[43,44]. A review of 25 cases of secondary cervical dystonia with a range of causes for the lesions in adults revealed that structural lesions of the brainstem and cerebellum were the most frequent cause of 'lesional' cervical dystonia (44%), with basal ganglia lesions accounting for less (24%) of cases[45]. There are documented cases in which successful removal of cerebellopontine angle tumours caused improvement of cervical dystonia[46]. 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[47]. 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[48-50]. Interestingly the important interplay between different brain regions has also been demonstrated within this literature. Tranchant et el detailed a case of a cervical dystonia secondary to a cavernous angioma in the right cerebellar hemisphere. Quantification of brain activity using PET revealed contralateral cortical and striatal hypometabolism suggesting that cerebellar pathways destined for these contralateral structures had been functionally interrupted[51].

Some but not all patients with genetic degenerative cerebellar disorders (for example spinocerebellar ataxia type 3 (SCA3) or dystonia with cerebellar atrophy (DYTCA)) demonstrate dystonia as part of their clinical phenotype or as the predominant movement disorder. However the 'dysfunction' of such disorders is often not isolated to the cerebellum and limits the ability to make inferences about neuroanatomical localisation from such patients. Moreover, as soon as the syndrome becomes dystonia *plus* either another movement disorder (such as myoclonus dystonia (DYT11)) or other neurological/systemic features (such as the dystonia observed in corticobasal degeneration[52]) neuroanatomical corollaries are particularly difficult to disentangle. A further caveat with the lesion method for establishing structure-function is that the lesion often results in dystonia after a delay of several weeks. This may suggest that dystonia arises from secondary adaptive responses to the lesion.

1.3.3 Neurosurgical evidence

One of the very first pubmed listed papers exploring the cerebellum and dystonia is a paper by the neurosurgeon Cooper detailing the effect of thalamic lesions on torticollis in the New England Journal of Medicine in the early 60s. At that time it had been established that pallidal lesions had favourable effects on limb and truncal dystonia but had a more limited effect on torticollis. He also felt that there was a limited response of torticollis to ablations (unilateral or bilateral) of the ventrolateral nucleus of the thalamus. He therefore advocated extending lesions posteriorly into ventroposterolateral, ventroposteromedial and centrum medianum nuclei to interrupt afferent fibers received from the cerebellum. When bilateral lesions were inflicted some documented cases exhibited dramatic improvement (see Figure 1-7).

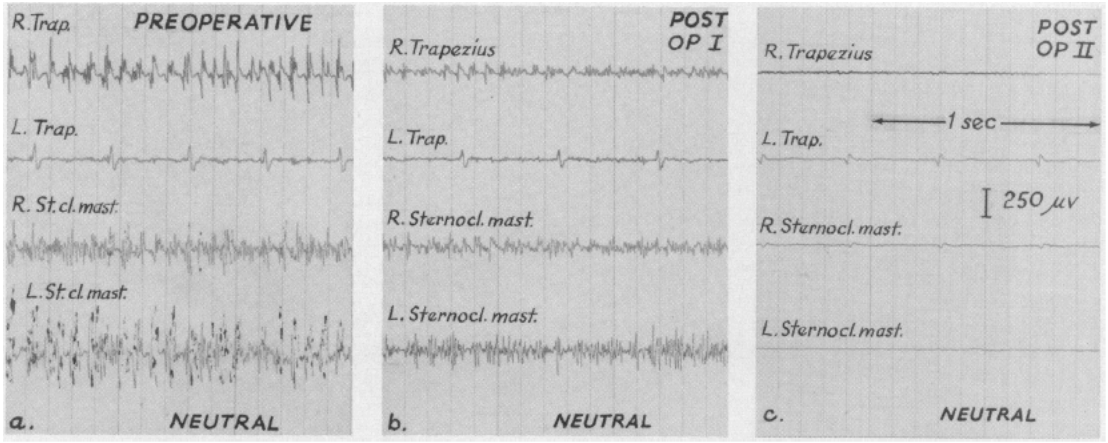


Figure 1-7 Effect of thalamic lesions on cervical dystonia

Example electromyographic traces obtained from a 33 year old man with torticollis: preoperatively (left panel), after right thalamic surgery (central panel – post op I) and after left thalamic surgery (right panel – post op II). Preoperatively he had severe torticollis with his occiput rotated to the left and his chin rotated to the right. The first operation was a right sided cryogenic lesion to the ventrolateral nucleus and anterior portion of the ventroposterolateral and ventroposteromedial nuclei of the right thalamus. There was immediate electrophysiological and clinical improvement bilaterally. Two weeks later he had a further cryogenic lesion to the left ventroposterolateral and ventroposteromedial nuclei with immediate 'abolition of all remaining involuntary muscular discharge'. At follow up one year later the patient was symptom free (figure and details from Cooper, 1964[53]).

Overall the effects of such thalamic surgery on greater numbers of patients were considered to be inconsistent but this is perhaps not surprising as precise neuroimaging was not always available and the full aetiological diversity of dystonia had not yet been appreciated. Formal studies comparing pallidal and thalamic deep brain stimulation have never been done but small numbers of cases do suggest that it may be safe and efficacious (particularly in subgroups with tremor (ventral intermediate nucleus) or forms which have a more limited response to pallidal surgery such as DYT6[54-56]. The exact site for stimulation although remains to be determined and some but not all of these studies targeted the nuclei receiving cerebellar afferents.

Others have focused more directly on the cerebellum. A variety of dyskinetic movements can improve following ablation or deep brain stimulation (DBS) of the dentate nucleus or superficial stimulation of the cerebellar cortex[57,58]. In addition for cervical dystonia the interstitial nucleus of Cajal (INC) in the midbrain reticular formation is a viable target. The INC is a core center for the control of head and neck posture intimately related to the cerebellum. Stimulation or destruction of the INC results produces abnormalities of head control in cats and monkeys and there are some reports of beneficial effects of lesioning the INC and nearby structures among patients with cervical dystonia[4].

1.3.4 Genetic insights

The distribution of expression of known genes for isolated dystonia provides a fascinating insight into a genetic neuroanatomy. DYT1 and DYT6 dystonia are both inherited in an autosomal dominant fashion with incomplete penetrance, due to mutations in TOR1A and THAP1 respectively. Both typically cause generalised dystonia although milder focal presentations are also recognised. More recently three additional genes are reported to account for a proportion of late-onset isolated cervical dystonia: CIZ1 (Cip1-interracting zinc finger protein; DYT23), ANO3 (anoctamin 3; DYT24) and GNAL (guanine nucleotide binding protein; DYT25)[59]. There are significant differences in the relative levels of expression for each of these genes (see Figure 1-8). For example in DYT6, the THAP1 gene is expressed most highly in the cerebellum. In contrast for the other 4 mutations cerebellar expression levels are lower relative to the other regions. Such patterns may suggest that there are subtle differences in the dystonia network at the root of pathophysiology for different genetic subtypes despite similar clinical movement disorders. Within the cerebellum expression is thought to be highly homogenous with virtually no differences in levels of expression between lobules and across individuals.

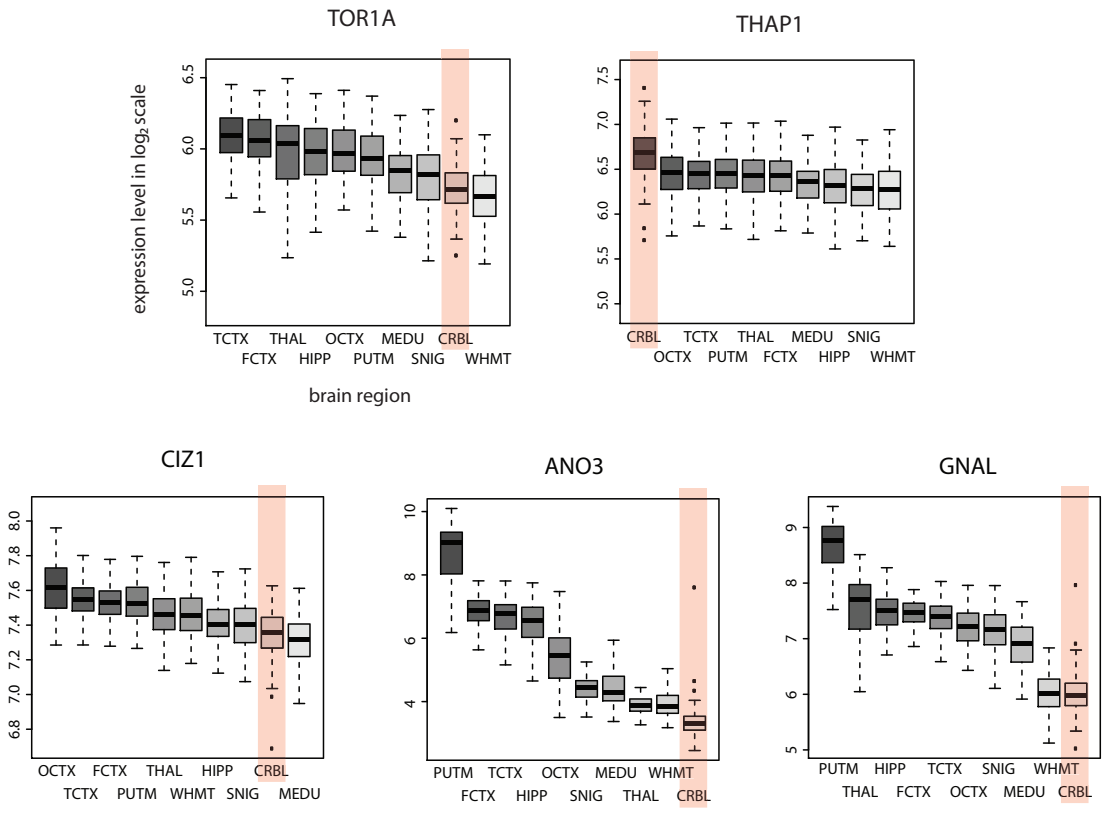


Figure 1-8 Genetic neuroanatomy

Boxplots of messenger RNA levels across ten different brain regions (x-axis). The expression levels are based on exon array experiments and are plotted on a log_2 scale (y-axis). The plot shows significant variation in transcript expression across the ten central nervous system regions analysed

(number of samples for each brain region given in brackets): cerebellar cortex (CRBL, n=130) - highlighted in red), thalamus (THAL, n=124), putamen (PUTM, n=129), substantia nigra (SNIG, n=101), inferior olivary nucleus in medulla (MEDU, n=119), frontal cortex (FCTX, n=127), temporal cortex (TCTX, n=119), occipital cortex (OCTX, n=129), hippocampus (HIPP) and intralobular white matter (WHMT). These images were generated using the web resource http://www.braineac.org from the UK Human Brain Expression Consortium

1.3.5 Histopathology

For many neurological disorders such as Parkinson's disease or Alzheimer's disease neuropathology has been instrumental in understanding aetiology and neuroanatomy. In contrast, for isolated dystonia only a few studies have been published and no well established morphological abnormalities have been described[60]. Clearly conclusions from such studies are limited by the resolution of the histological method applied, and more sophisticated methods may reveal more subtle defects. However the general paucity of overt structural defects may suggest that dysfunction in isolated dystonia is functional in nature and until its specific substrate is know may remain concealed from histopathological analysis [61].

1.3.6 Human neuroimaging

Imaging studies in isolated dystonia have been extensively reviewed recently (see Neychev et al., for full tabulation of study outcomes by imaging modality and dystonia subtype[4]). Imaging modalities are attractive with increasing ability to detect subtle abnormalities in structure or function in dystonia.

One of the most widely cited lines of cerebellar evidence is work by the Eidelberg group. Diffusion tensor-imaging (DTI) magnetic resonance imaging can be used to analyse the integrity of white matter tracts. In both DYT1 and DYT6 reductions in connectivity involving the cerebello-thalamic projections were observed in mutation carriers whether or not they exhibited signs and symptoms of the disorder. Furthermore disruption of the cerebellar outflow are linked to abnormally elevated activity of the regions of the sensorimotor network required for the task (as defined by multivariate analysis of functional imaging[62]). A second fibre tract abnormality involving thalamocortical projections was also identified for non-manifesting gene carriers. Counter intuitively it is this second 'lesion' which is hypothesized to block the effect of the cerebello-thalamic lesions or the expression of dystonia[62-67]. Interestingly this idea that abnormal cerebellar outflow drives dystonia was not upheld in the DYT1 knock-in mouse detailed above which was designed to examine this neuroimaging model of dystonic pathophysiology.

Overall imaging studies point to dysfunction across a range of brain regions (rather than a single region). However statements such as 'the cerebral cortices, basal ganglia, thalamus,

cerebellum and brainstem are the most frequently implicated regions in dystonia' are not uncommon in this literature. This is most of the brain and does not provide us with much useful pragmatic information about how to better investigate and treat dystonia.

Another limitation of these studies is the lack of consistency among different studies. For example, a summary of studies which have examined cerebellar grey matter volume in dystonia using voxel based morphology is given in Table 1-1. Both increases and decreases of cerebellar grey matter volume have been found, sometimes changes are limited to specific areas of the cerebellum sometimes not, sometimes changes are unilateral, sometimes they are bilateral.

Type of dystonia	Case/controls	Cerebellum	Source
blepharospasm	20/11	↑ left and right hemisphere	[68]
spasmodic	40/40	↑	[69]
cervical	10/10	↓ flocculus	[70]
craniocervical	35/35	↑ anterior hemisphere anteriorly, ↓ post hemisphere posteriorly	[71]
craniocervical	27/54	↓ vermis & left hemisphere	[72]
cervical	9/11	↑ left hemisphere	[68]
writing dystonia	22/28	no change	[73]
writing dystonia	30/30	↓	[74]
mixed focal dystonia	45/22	↑ hemisphere	[75]

Table 1-1 Cerebellar grey matter volume in isolated dystonia.
An upward going arrow is used to indicate an increase in cerebellar volume and a down going arrow to indicate a decrease in cerebellar volume.

Moreover when focusing on specific regions of interest it is very difficult to distinguish cause from effect. Dysfunction of one brain region responsible for triggering dystonia is likely to lead to downstream secondary effects in other regions. Additionally dystonic movement itself may result in structural or functional changes in the brain, epiphenomena related to the dystonic movement not causal to pathophysiology. It is likely that modeling of patterns of covariance among different regions may provide a more relevant measure for dystonia than isolated increases or decreases in a specific region. Such approaches after all look to define a dystonic network which appears to be a more accurate reflection of the disease.

1.3.7 Electrophysiological studies

Impaired inhibitory mechanisms and maladaptive plasticity are hallmark neurophysiological findings across subtypes of dystonia. Impaired inhibition has been demonstrated at multiple levels of the central nervous system[76] and there is evidence for impaired regulation of plasticity mechanisms in both human and animal studies[77]. Interestingly cerebellar disorders classically have a contrasting neurophysiological profile. Increased levels of inhibition are suggested by findings such as prolongation of the cortical silent period and increased motor cortical threshold although plasticity inducing paradigms may be normal[78,79]. There is likely to be variability of such markers across subtypes of genetic cerebellar disroders[80] suggesting that there are differences in such processes despite a similar motor phenotype. However, overall shifts in neurophysiology are the converse to those associated with isolated dystonia.

Some studies have more directly explored the relationship between the cerebellum and dystonia pathophysiology. For example a paradigm called cerebellar brain inhibition (CBI) examines the cerebello-thalamo-cortico pathway. In healthy subjects, a conditioning pulse delivered over the cerebellar cortex 5–7ms prior to a test pulse over the contralateral primary motor cortex will result in reduction of the motor evoked amplitude relative to a test pulse given alone over this cortical area. 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[81]. In eight patients with idiopathic focal limb dystonia, cerebellar brain inhibition was initially thought to be reduced[82]. However more recently this finding was not reproduced on a follow up study with the same patient group[83].

The most compelling electrophysiological lines of evidence for cerebellar involvement in dystonia is seen when studying eye blink conditioning. As detailed already there is good evidence that this type of associative learning is encoded within the cerebellar circuitry[30]. Reassuringly patients with Parkinson's disease perform as well as healthy controls indicating that basal ganglia dysfunction does not necessarily impact on this learning paradigm[84]. In contrast, patients with adult onset focal dystonia (task-specific and cervical dystonia) have reduced levels of conditioning[85].

Again there are difficulties with commonly applied physiology methods for localisation of functional anatomy of dystonia as any change presumably reflects an emergent property of interactions between multiple regions within the dystonic network[4]. As such abnormalities may reflect a core defect underlying a predisposition to develop dystonia, a downstream consequence of the motor disorder or even a phenomenon of little relevance (epiphenomenon)[4].

1.3.8 Behavioural studies

Another line of investigation examining cerebellar function in dystonia has been the study of motor tasks which require intact cerebellar function. In DYT1 dystonia, both manifesting and non-manifesting subjects are impaired in sequence learning in which the sequential order of targets is learnt [86,87] and functional imaging demonstrated over-activity of the left cerebellar cortex (whilst subjects moved the right arm)[87,88]. However sequence learning recruits many brain regions including the basal ganglia, and the over-activation of the contralateral cerebellar hemisphere to hand movement makes the functional significance of these findings in dystonia difficult to gauge. Recently it has been shown that sequence learning is normal in cervical dystonia[89].

1.4 Interpretation

In summary it can be seen that the cerebellum has a rather unique microstructure which may indicate that it performs a common computation across multiple domains which include motor control and cognition. There are experimental paradigms which across species have been shown to require the cerebellum and components of such paradigms have been directly mapped to the cerebellar microstructure. Despite much research which has supported the idea that dystonia represents a network disorder it has remained elusive to try and define how genetic mutations translate into the movement disorder we observe in clinical practice. It is relatively recently that the cerebellum has been considered an important node within this network and there is little research exploring its mechanistic function. Defining the functional role of the cerebellum in the pathophysiology of dystonia

1.5 Aims of thesis

The overall aim of this thesis was thus to design and conduct experiments that better defined the functional role of the cerebellum in the pathophysiology of dystonia.

In chapters 2 and 3 I used cerebellar direct current stimulation to modulate the cerebellum activity in order to investigate the cerebellar contribution to two of the electrophysiological hallmarks of dystonia; abnormal surround inhibition and abnormal plasticity regulation. Given the evidence pointing to a role for the cerebellum in the pathophysiology of dystonia our hypothesis was that the cerebellum would be able to modulate either or both of these electrophysiological paradigms with a potential therapeutic role ensuing if either of these studies were positive.

In chapter 4 I examine eye blink conditioning in the monogenic DYT1 and DYT6 dystonias. As outlined in the introductory section, this is one of the purest cerebellar paradigms available and if impaired would give substantial insight into cerebellar mechanisms.

In chapter 5 I explored the role of millisecond timing and whether or not this is impaired in isolated dystonia. If a timing deficit was at the root of elevated temporal discrimination thresholds in dystonia this may be indicative of abnormal cerebellar function.

In chapter 6 I assessed the archetypical motor cerebellar paradigm, adaptation, to evaluate if it was impaired in patients with cervical dystonia. I also examined the relationship between rates of adaptation and features of dystonic head tremor. In the last experimental chapter, I tested adaptation in DYT1 dystonia to see whether imaging work showing reduced cerebello-pontine-thalamic pathway integrity could be corroborated by behavioural findings supporting an adaptation deficit.

The results are then collated in the final chapter before making tentative conclusions about the role of the cerebellum in the pathophysiology of dystonia and outlining directions for future work.

2 Cerebellar modulation of motor surround inhibition

2.1 Introduction

Surround (or lateral) inhibition is a term used to describe multiple phenomena throughout the nervous system in which neural signals to a central receptive field or target are facilitatory and eccentric signals are inhibitory[90]. Within the motor system, it was first explored conceptually as a mechanism by which basal ganglia circuits selectively execute desired motor programs. Later, a potential neurophysiological measure motor surround inhibition (mSI) was demonstrated; by stimulating the motor cortex using transcranial magnetic stimulation (TMS) at the onset of movement of the index finger, suppression in the size of responses of non-synergistic surround muscles was seen[91].

It is not known which neuroanatomical structures within the central nervous are important for the generation of mSI. Some authors favor a neocortical mechanism following the observation that hemispheric dominance and task difficulty modulate the magnitude of mSI[92]. However electrophysiological studies examining the dependency of mSI on dorsal and ventral premotor and motor cortex interactions have failed to support this notion[93,94].

The cerebellum plays a major role in temporal encoding and coordination of movements and deficiencies in hand control and individual finger movements are seen in patients with cerebellar disease[95]. It also has a net inhibitory effect on the cerebral cortex via the cerebello-dentato-thalamo-cortical pathway[95,96]. These characteristics make the cerebellum a suitable candidate to functionally contribute to the generation of mSI.

Previous work examining cerebellar brain inhibition (CBI) and individual finger movements demonstrated a nonspecific decrease in cerebellar inhibition to active and surround muscles at the motor cortex at the onset of movement but no link between mSI and CBI[61]. However CBI relies on a powerful (and painful) phasic non-topographically specific magnetic stimulation of the cerebellum which may not reveal subtle changes in paradigms such as mSI. In this study we utilise transcranial direct current stimulation of the cerebellum (cDC) which has emerged as an important technique by which to enhance (anodal) or decrease (cathodal) cerebellar excitability[24]. The cerebellum is stimulated for 15 minutes and changes in excitability are seen for at least 30 minutes. This has been confirmed neurophysiologically (measuring CBI) and behaviourally (measuring rates of adaptation to sensory perturbations, a cerebellar dependent learning task); anodal cDC increases CBI and leads to faster rates of adaptation and cathodal cDC decreases CBI[24]. In addition cDC can be used to assess the cerebellar contribution to neurophysiological paradigms; recently the cerebellum was shown

to be a critical structure for the generation of motor cortex plasticity responses to paired associative stimulation with an inter-stimulus interval of 25ms[97].

2.2 Methods

Twelve right-handed healthy subjects (mean age: 25 yrs, range: 19-35 yrs, 9 male) with no history of neurological or psychiatric disease participated in the study. Written informed consent was obtained from all participants and the study was approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki 2008.

Disposable surface EMG electrodes were placed on the right first dorsal interosseus (FDI) and abductor digiti minimi (ADM) muscles using a belly-tendon montage. The signal from the EMG electrodes was amplified (gain 1000), bandpass filtered (20–2000 Hz) (Digitimer D360 amplifier) and digitized at a sampling rate of 5 kHz and stored in a laboratory computer for off-line analysis by CED 1401 hardware and Signal software (Cambridge Electronic Design Ltd).

Monophasic TMS pulses were delivered from a Magstim 200 stimulator. A figure-of-eight coil (external loop diameter of 9cm) was held tangentially on the scalp at an angle of 45° to the midsagittal plane with the handle pointing laterally and posteriorly. Corticospinal tract excitability was measured as the peak-to-peak amplitude of the motor evoked potential (MEP) generated by single pulse TMS. TMS was applied to the motor “hot-spot” of the right ADM muscle that was defined as the point where a magnetic stimulus of slightly suprathreshold intensity consistently elicited a MEP in ADM of the highest amplitude. This position was marked on a tight fitting neoprene cap in order to ensure consistent coil position during the experiment.

cDC was applied to the cerebellum as described previously[98]. It was delivered with an intensity of 2mA using a direct current stimulator through 25 cm^2 saline-soaked surface sponge electrodes (Eldith-Electro-Diagnostic & Therapeutic Systems GmbH, Germany). One electrode was centred on the right cerebellar cortex, 3cm lateral to the inion and the other electrode was positioned on the right buccinator muscle. Anodal or cathodal cDC was delivered over the cerebellum for 15 min. In the sham session, anodal cDC was applied for 30s in order that a true sham condition was simulated (some subjects experience tingling at site of electrodes when stimulation is initiated). At the onset and offset of all interventions (anodal, cathodal, and sham) current was changed in a ramp-like manner over 10s. Subjects were supervised during cDC and listened to a radio documentary. They were asked to keep all movement, specifically finger movements, to a comfortable minimum.

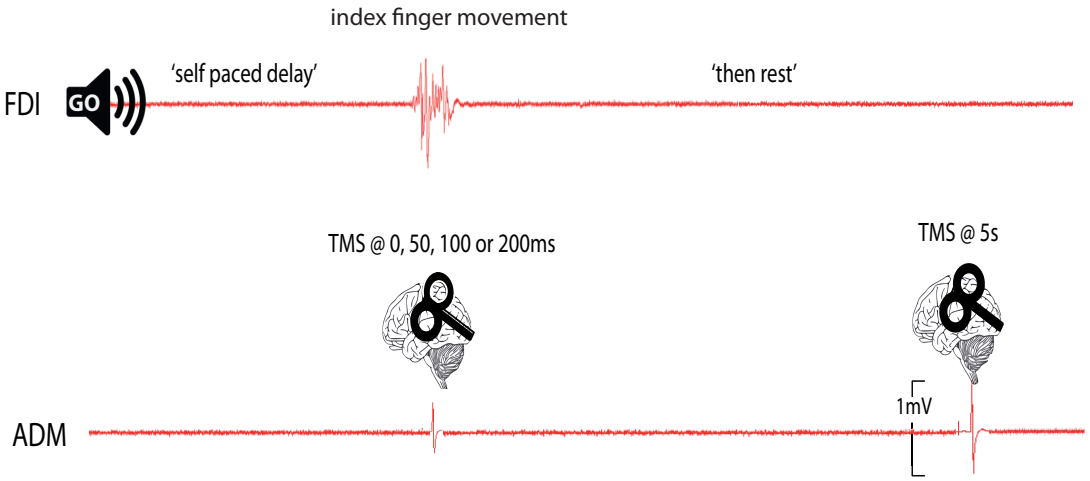


Figure 2-1 Experimental method to assess mSI

Five states of self-triggered TMS were applied in a random order at variable intervals between EMG onset and TMS trigger (0ms, 50ms, 100ms, 200ms and 5s).

Subjects were seated in a chair with their right hand resting in a relaxed position on a desk. They were asked to briefly depress a small button with the index finger after a 'go' signal (an auditory tone of 50ms) with a self-paced delay (Figure 2-1). FDI is a synergist rather than a primary muscle for this movement and previous studies have shown that this movement induces activation of FDI and suppression of the MEPs elicited in the ADM muscle[91]. Subjects were first asked to press with maximal force, and amplitude of mean EMG activity in FDI was noted. Subjects were then trained to perform the movement to the amplitude of 10% maximal EMG activity while visual feedback of the muscle activity was projected on a screen in front of them. Duration of the movement was approximately 100ms. We favoured a short movement duration to facilitate production of a clean onset and offset of EMG activity as mSI has been found to be active only during the initiation of the movement and not later during tonic muscle contraction[91]. Subjects were also asked to keep the surround muscle ADM relaxed while they were performing the movement. Training was continued until subjects achieved consistent performance of the desired movement and raw EMG signal in ADM muscle was not in excess of 100 μV.

Each subject took part in a cross over study, which consisted of each of the three types of stimulation (sham, cathodal or anodal) in a randomised order. Each session was separated by a week. Resting motor threshold (RMT) was measured and was defined as the lowest intensity (expressed as a percentage of maximum stimulus intensity (MSO)) that evoked a response of about 50µV in the relaxed ADM in at least five of ten trials[99]. The intensity of the

stimulation was then set to evoke ADM MEPs with average peak-to peak amplitude of approximately 1mV at rest for the remainder of the experiment.

For the assessment of mSI, five states of self-triggered TMS were applied in a random order at variable intervals between EMG onset and TMS trigger (0ms, 50ms, 100ms, 200ms and 5s). This allowed us to assess the magnitude of mSI at time 0ms and also assess if cDC induced changes in the timing profile of inhibition/mSI at later time intervals. The TMS pulse was triggered when EMG signal of right FDI rose above 100μV. 20 trials of 5s (rest) and 15 trials of the other 4 intervals (0ms, 50ms, 100ms and 200ms) were collected. Five seconds after the onset of movement is considered to be sufficient for measurements at rest as no post activation inhibitory or facilitatory effect are known to be active at this time[91].

For each subject peak-to-peak MEP amplitude for each trial was measured off-line and the mean MEP amplitude at rest and at each time interval was calculated. For each interval, mean MEP amplitude was then divided by mean rest MEP amplitude for the respective muscle (labeled in graphs as % resting MEP). If the ratio is less than one, there is evidence for mSI. When it is greater than or equal to one, there is no mSI.

Unless otherwise stated all results are expressed as mean ± standard error of the mean (SEM). We used SPSS software (version 19) for statistical analysis. Kolmogorov-Smirnov test was used to explore the normality of the data distribution and Levene's test was used to explore the homogeneity of variance. Log10 transformation was performed when data were not normally distributed.

Repeated measures analysis of variance (rmANOVA) was used to confirm the presence of mSI in ADM and to assess the effects of cDC on the magnitude of mSI before and after stimulation. Bonferroni's correction for multiple comparisons was used for post hoc t-tests. In order to quantify intrasubject and intersubject variability the coefficient of variation (COV) was expressed as a percentage. The COV is the ratio of the standard deviation to the mean.

2.3 Results

All subjects completed the three sessions without any adverse events and each experimental session lasted two hours.

2.3.1 Baseline measures

The mean stimulus intensity for RMT of ADM across the 3 sessions for all subjects was 41% of MSO (±2.3%). The stimulus intensity required for a 1mV MEP in ADM ranged from 38% to 80% of MSO across subjects with a mean value of 57% (± 3.4%). The mean stimulus intensity required for a 1mV MEP in ADM was 137% of the RMT.

2.3.2 mSI present in ADM

Figure 2-2 demonstrates the profile of MEP sizes in the FDI and ADM muscles for each of the intervals tested. MEPs are expressed as % resting MEP and the group mean is derived from the individual mean of the 3 baseline measurements of mSI taken at each session. Log_{10} transformation was performed and the data satisfied the assumptions for parametric tests after the transformation. One-way rmANOVA revealed a significant effect of INTERVAL (0ms, 50ms, 100ms and 200ms) in the ADM muscle $F(3,7)=22.84$, $p<0.001$ and FDI muscle $F(3,7)=15.84$, $p<0.001$.

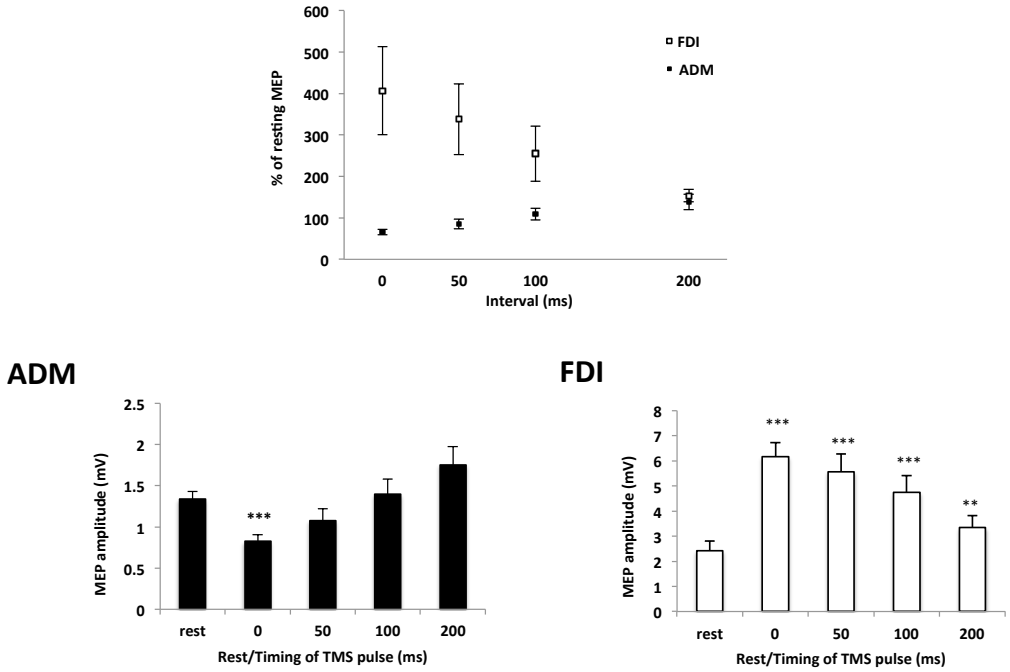


Figure 2-2 Profile of mSI

*This figure demonstrates the group mean of the individual means across the three baseline sessions. In the upper panel the normalised data are shown for both muscles. Raw MEP data is given for individual muscles below. The surround muscle ADM is significantly inhibited at time interval 0ms. Note the reduction of variability in the ADM muscle MEPs (as indicated by the error bars demonstrating the standard error). The active muscle FDI is facilitated at the onset of movement and the later time intervals tested (** p ≤0.01; *** p ≤0.001).*

In ADM post hoc paired sample t-tests of raw MEP data at rest (5s) and during movement (0ms, 50ms, 100 ms and 200 ms) revealed that mSI was present at time 0ms, thus MEPs in ADM were significantly inhibited at time interval 0ms $t(11)=4.93$, $p<0.001$. There was no significant inhibition of ADM at the other time intervals and it can be seen from Figure 2-2 that the MEP size gradually increases. Only one subject had a mean ADM MEP amplitude at the onset of the movement (0ms) which was not inhibited compared to the resting MEP (mean mSI = 1.12 ±0.04 across three baseline sessions). The MEP was still suppressed in

this subject (as there is an increase in spinal excitability at 0ms[91] but it is not by definition inhibited).

In FDI there was significant enhancement of MEP amplitudes at all of the time intervals (0ms, 50ms, 100ms and 200ms) compared to rest (0ms: $t(11)=-8.77$, $p<0.001$; 50ms: $t(11)=-5.46$, $p<0.001$; 100ms: $t(11)=-4.27$, $p=0.001$; 200ms: $t(11)=-3.45$, $p=0.005$).

2.3.3 Effect of cDC on mSI

In order to explore the effect of cDC on mSI we looked at the magnitude of mSI at 0ms in the muscle ADM at each of the time points measured (baseline, T0, T20). Repeated measures ANOVA with factors TIME (baseline, T0, T20) and cDC (sham, anodal, cathodal) revealed no significant effect of TIME ($F(2,10)=1.09$, $p=0.35$), cDC ($F(2,10)=1.03$, $p = 0.38$) or their interaction ($F(4,8)=1.05$, $p=0.39$). There was also no significant effect of cDC on MEP profile at any of the other intervals tested (50ms, 100ms or 200ms) (Figure 2-3 B-D). Based on these results we conclude that the cerebellum does not seem to have a role in the generation of mSI.

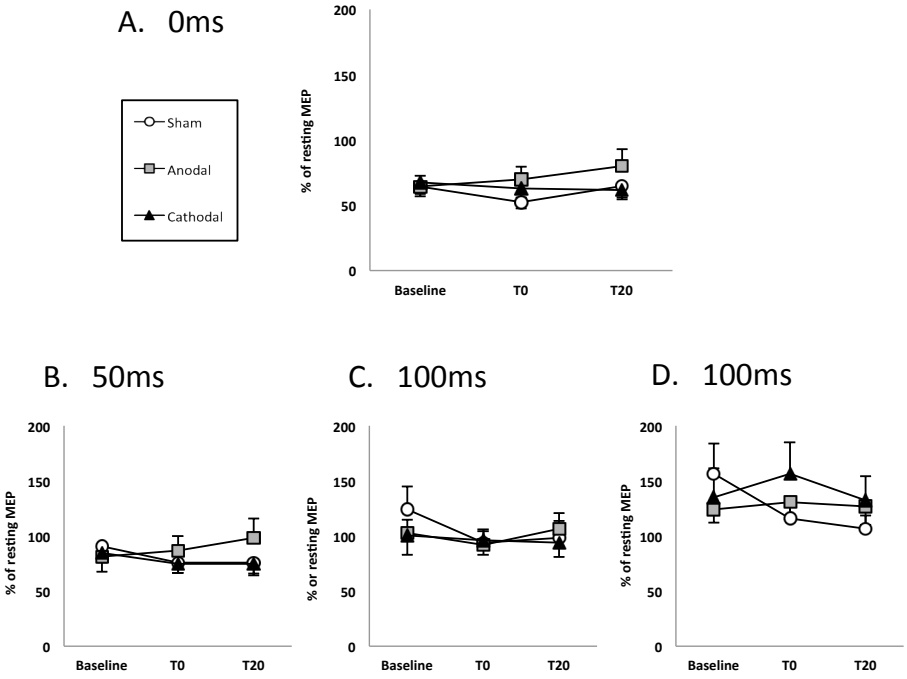


Figure 2-3 Effect of cDC on mSI

There was no significant modulation of the magnitude of mSI by cDC.

2.3.4 Intrasubject and intersubject variability of mSI

In order to quantify variability of mSI we examined mSI seen in ADM at the onset of index finger movement (interval 0 ms). Intrasubject variation of mSI (range of mSI responses

exhibited by a single subject) as assessed by COV had a mean value of 27% (range from 14% to 48%). Intersubject variability (different subjects) had a mean value of 44% (range from 40% to 46%) (see Table 2-1).

2.4 Discussion

Motor surround inhibition was clearly demonstrated across subjects; at 0 ms there was consistent and statistically significant inhibition of MEPs in ADM. The study design allowed three measures of mSI on different sessions in the same subjects and mSI was confirmed to be stable within subjects. Given the intrinsic variability of MEPs this marks out the measurement of mSI a robust and reproducible TMS paradigm. This is in contrast to some other commonly used electrophysiological paradigms such as paired associative stimulation (PAS) which I will go on to discuss in Chapter 3 and emphasises the potential importance of the deficiency of mSI seen in diseases of motor control such as focal hand dystonia and Parkinson's disease[100]. Attempting to modulate the strength of mSI, as in this study, therefore was an important potentially therapeutic goal in neurophysiological studies of mSI

mSI is defined as the functional inhibition of surround muscles seen during the movement initiation phase (and just before and during the first phase of EMG onset)[100]. The mechanisms of how and where it is generated are less well characterised. At the spinal level there is a non-spatially selective facilitation at these time points (shown by F-wave and H-reflex studies) and thus mSI is thought to reflect a supra-spinal control mechanism[91]. We find no evidence that modulating the excitability of the cerebellum in isolation can change the magnitude of mSI. This adds to previous work examining CBI which did not find a functional link between mSI and CBI[96]. In addition no association between activity in premotor cortex (both ventral and dorsal) and mSI has been demonstrated[93,94]. It may be that mSI is a fundamental inhibitory mechanism within the nervous system and subtle alteration of the activity of one of the nodes within the mSI network does not allow a meaningful change in mSI to be observed. Alternatively the genesis of mSI may reside within other areas such as the basal ganglia nuclei. It should be possible in the future to explore this hypothesis by measuring mSI in patients with Parkinson's disease or dystonia before and after deep brain stimulation.

At the synaptic level a GABA-ergic mechanism for mSI has been proposed largely based on animal work[100]. In humans, proving the link between GABAergic circuits and mSI is less certain. No functional link has been shown between mSI and short-interval intracortical inhibition (SICI) and cortical silent period, which are indirect markers for $GABA_A$ and $GABA_B$ receptor function respectively[100,101]. Other inhibitory projections to M1 are reduced at the onset of movement and do not consistently demonstrate the action specific modulation of

muscle excitability unique to mSI (long-interval intracortical inhibition, short-latency afferent inhibition, long-latency afferent inhibition, interhemispheric inhibition, CBI)[96,102,103].

Subject	1	2	3	4	5	6	7	8	9	10	11	12	Intersubject COV (each session)	Mean Intersubject COV
mSI (session 1)	0.29	0.39	0.47	0.56	0.62	0.42	0.84	0.94	0.41	1.02	0.80	0.94	40%	44%
mSI (session 2)	0.46	0.42	0.72	0.37	0.44	0.66	0.65	0.51	1.17	0.56	0.52	1.26	45%	
mSI (session 3)	0.35	0.31	0.26	0.53	0.46	0.56	0.78	0.82	0.81	0.89	1.20	1.14	46%	
Mean mSI for each subject	0.37	0.37	0.48	0.49	0.51	0.55	0.76	0.76	0.80	0.82	0.84	1.12		
Intrasubject COV (individual values)	24%	15%	48%	22%	19%	22%	13%	29%	47%	28%	41%	14%		
Mean (n=12) Intrasubject COV	27%													

Table 2-1 Intrasubject and intersubject variability of mSI.

Intrasubject and intersubject variability of mSI exhibited in ADM muscle at the onset of movement (interval 0 msec). Values are shown for each session before any stimulation. Each measure of mSI is given as a ratio of mean resting MEP for ADM (normalised values). Intrasubject and intersubject variability are compared using the coefficient of variation (COV).

There is increasing evidence that mSI is an adaptive phenomena. It has previously been shown that mSI is more pronounced in the dominant hemisphere, is stronger with low force levels, and starts earlier with increasing task difficulty[92,104]. More recently it has been demonstrated that the magnitude of mSI is increased by carefully timed vibration training[103,105]. Conversely, 30 minutes of finger exercises with synchronised movements of the index and little finger in contrast to little finger movements alone, reduces the magnitude of mSI, perhaps blurring individuation of digits as measured by mSI or implicating a role for fatigue on mSI modulation[106].

The failure of cDC to modulate mSI was surprising. We believe cDC to be an excellent tool to explore the functional network that contributes to mSI; indeed in the visual cortex anodal cDC has recently been found to change surround suppression, a comparable paradigm to mSI in the visual system[107]. It is an interesting question whether the degree of adaptation of mSI may be increased or decreased by stimulation techniques; one might expect cDC to modify the adaptation seen with vibration training.

Further characterisation of mSI remains a challenging field. It is worth restating that the first study of mSI found comparable amounts of inhibition in ADM when the paradigm is triggered by mouth or leg movement (risorius: 77%; tibialis anterior: 68%)[91]. This finding has never been replicated but suggests a less spatially specific mechanism for mSI than is currently discussed, particularly when mSI is mentioned in the context of models of focal hand dystonia. Additionally the current literature freely moves between using the term surround inhibition as a cellular mechanism in the senses, neurophysiological mechanism in motor (mSI) and sensory systems (somatosensory evoked potentials[108]), as a mechanism for

selecting motor programmes[109] and as an explanation for psychophysical phenomena[110]. To move away from a purely descriptive term that represents the capability of organisms to attach saliency to inputs or produce specific commands, we must examine the similarities and differences between surround inhibition at each hierarchical level and modality to understand its mechanisms further.

A limitation of our study is that subtle differences in experimental conditions across the three sessions may have lead to incorrect acceptance of the null hypothesis that the cerebellum does not functionally contribute in the generation of mSI (both subject dependent e.g. level of attention to task and experimental e.g. differences in placement position of TMS coil). We considered increasing the number of subjects but as no trend was seen in our twelve subjects we consider the acceptance of the null hypothesis to be correct.

We find mSI to be a robust electrophysiological phenomena with minimal intrasubject variability over the three sessions in this study. Quantification of intrasubject variability in this study will allow future therapeutic studies that attempt to modulate mSI to be adequately powered. We do not find evidence to suggest that the cerebellum contributes to the neuroanatomical network necessary for the generation of mSI. We have reviewed the current literature on mSI and identify important future challenges in the field that need further investigation so that the physiology of mSI and its deficit in certain diseases is more clearly understood.

3 Cerebellar modulation of motor cortex plasticity in writing dystonia

3.1 Introduction

Despite increasing understanding of the pathophysiology of dystonia, defining novel treatments based on research findings has remained elusive. In parallel to work suggesting a role for the cerebellum in the pathophysiology of dystonia, multiple electrophysiological studies demonstrate reduced inhibition throughout the central nervous system. Specifically reduced *cerebellar* inhibition of the *motor cortex* is suggested by the finding of reduced cerebellar brain inhibition (CBI) in focal dystonia (CBI tests the functional integrity of the cerebello-thalamo-cortical pathway)[82]. In addition, abnormal plasticity regulation has been demonstrated and responses to plasticity protocols are widely considered to be excessive and non-selective in the motor cortex and other sites within the dystonic network[111].

When we (and others) found that plasticity responses of the motor cortex could be reduced by cerebellar stimulation in healthy subjects it was an intriguing hypothesis to explore whether the excessive plasticity responses described in dystonia could be normalised by cerebellar stimulation[97,112]. We chose to give patients with writing dystonia anodal cerebellar transcranial direct current stimulation (cDC), as this was the type of stimulation with the greatest block of plasticity in healthy subjects[97]. In addition anodal cDC is thought to functionally increase cerebellar activity, increasing cerebellar inhibition of target structures, and thus is the intuitive choice in a disease in which the motor cortex is hyper-excitable. Proof of the concept that modulation of cerebellar activity is beneficial could provide exciting new treatment options for dystonia.

3.2 Methods

Ten patients with writing dystonia diagnosed at the National Hospital for Neurology and Neurosurgery, London were recruited (Table 3-1). All completed a two-part study (sham and anodal cDC) in which cDC was given simultaneously to paired associative stimulation (PAS25). The first cDC stimulation type was randomised and the patients were blinded. Experimental sessions were performed a week apart at the same time. Written informed consent was obtained and the study approved by the local ethics committee. No patients had contraindications to transmagnetic stimulation (TMS)[113]. All subjects completed the experiments without complications.

Sex	Age	Hand	Years of dystonia	Overflow	Tremor	Last botox	Descriptive Phenotype
F	55	R	14	++	-	5 y	Slow effortful writing, wrist extension and elbow elevation
M	42	R	7	-	-	3 y	Finger and thumb flexion with excessive grip strength
M	50	R	7	-	-	2 y	Involuntary extension of thumb. Holds pen with index and middle finger
M	57	R	12	++	-	6 m	Abnormal finger flexion with increased grip of pen
F	52	R	15	-	-	4 y	Abnormal thumb flexion
M	49	R	26	+	Yes	-	Abnormal flexion of fingers
M	58	R	20	++	Yes	10 y	Abnormal grip with involuntary flexion of fifth digit and wrist flexion.
M	66	R	8	-	-	-	Abnormal flexion of fingers
M	64	R	24	+	Yes	6 y	Abnormal thumb extension and wrist ulnar deviation and extension
M	35	R	11	-	-	5 y	Abnormal flexion of thumb and index finger

Table 3-1 Patient characteristics.

Key to abbreviations: Hand Handedness assessed by Edinburgh Handedness Inventory; R right; L left; Overflow '-' no overflow to tasks other than writing; '+' one other task; '++' multiple other tasks; Last botox last botulinum toxin injection given in either m months or y years; '-' has never received botulinum toxin injections.

3.2.1 PAS

PAS consisted of 180 electrical stimuli of the right median nerve at the wrist paired with a single TMS over the hotspot of right APB muscle at a rate of 0.2Hz. Electrical stimulation (square wave pulse; stimulus duration, 0.2ms) was applied at an intensity of three times the perceptual threshold using a constant current generator (Digitimer). TMS was applied at a stimulus intensity (SI) required to elicit a mean motor-evoked potential (MEP) of amplitude 1mV. The interstimulus interval between peripheral and TMS stimuli was 25ms (PAS25). This protocol has previously been shown to induce a long lasting increase in MEP amplitude[97]. Subjects were instructed to look at their stimulated hand and count the peripheral electrical stimuli they perceived.

3.2.2 TMS and EMG

TMS was delivered by a Magstim 200^2 stimulator (Magstim Company, UK) every 4.5-5.5 sec. A figure-of-eight coil (outer winding diameter 70 mm) was held tangentially on the scalp at an angle of 45° to the midsagittal plane with the handle pointing laterally and posteriorly. Motor cortex excitability was measured as the peak-to-peak amplitude of the MEP generated by a single TMS pulse. TMS was applied to the motor cortex representation of the right abductor pollicis brevis (APB) muscle. The motor hot spot was defined as the point where a magnetic stimulus of constant, slightly suprathreshold intensity consistently elicited an MEP of the highest amplitude. Surface EMG electrodes were placed over the right APB, first dorsal interosseous (FDI) and abductor digiti minimi (ADM) in a belly-tendon montage. Signal from EMG electrodes was amplified (gain 1000), bandpass filtered (20 Hz–3 kHz), digitized at a

frequency of 5 kHz, and stored in a laboratory computer for later offline analysis using Cambridge Electronic Design (CED) 1401 hardware and Signal software (CED).

3.2.3 cDC

cDC was applied to the cerebellum as described in section 2.2[98] *simultaneously* with PAS25[97]. Anodal cDC was delivered over the cerebellum for 15 min. In the sham condition anodal cDC was applied for 30 s. At the onset and offset of both interventions current was decreased in a ramp-like manner (over 10s).

3.2.4 Outcome parameters

Resting motor threshold (RMT) was defined as the lowest intensity that evoked a response of about 50μV in the relaxed APB in at least five of ten trials[113]. Active motor threshold (AMT) was defined as the lowest intensity that evoked a small response (>100μV) in more than five of ten consecutive trials when subjects maintained a slight contraction of the right APB (~10% of the maximum voluntary contraction)[113]. Stimulus intensity (SI) was changed in steps of 1% of the maximum stimulator output. Motor cortex excitability was measured with the SI required to elicit a 1mV MEP in APB at baseline and the SI was kept constant to measure post PAS25 response. Mean MEP amplitude of the 30 MEPs (30MEP) was calculated at each time point for all muscles recorded (APB, FDI and ADM). Ten MEPs each were recorded at 100, 120, 140, 160 and 180 % of the RMT for RC and the mean amplitude calculated for each SI to provide RC plots for all muscles. Linear regression analysis of the RC of *each* patient was performed (*r*RC) for data points between 100 and 140% of the RMT as described by others for group data[114] and a group mean calculated. Measurements of cortical silent period (CSP) duration were made during voluntary contraction of APB (20% of maximal voluntary contraction). SI was 120% RMT and 12 stimuli were given at a frequency of 0.2Hz. CSP was measured off-line for individual trials and defined as the duration from the onset of the MEP until the rectified EMG signal crossed the pre-stimulus mean activity. Patients had RMT, AMT, 30MEP, RC, and CSP recorded before (baseline) and after (T0 and T30) PAS25. Patients were videoed at the baseline and end of experiment.

Assessors blinded to cDC type scored patients with the writing movement sub-score of the Writer's Cramp Rating Scale (WCRS) [115]and the time taken to copy a standardised sentence (s). Patients rated change in symptoms using a visual analogue scale (VAS from -100% (deterioration) to +100% (resolution)).

3.2.5 Statistical analysis

To assess effect of cerebellar stimulation RMT, AMT and CSP (for APB) and 30MEP and rRC (for ABP, FDI, ADM) were evaluated by rmANOVA with factors 'cDC' (sham-PAS25, anodal-PAS25), 'TIME' (baseline, T0, T30) and 'cDC × TIME'. Patients were divided into

facilitators to PAS25 (amplitude of 30MEP larger at T30 compared to baseline, >0) and inhibitors to PAS25 (amplitude of 30MEP smaller at T30 compared to baseline, <0) for each muscle in the sham condition. Change in amplitude at T30 for sham and anodal cDC were compared in both groups using paired *t*-tests.

3.3 Results

RMT, AMT and CSP (for APB) and 30MEP and *r*RC (for ABP, FDI, ADM) did not change with the factors 'cDC', 'TIME', and we did not find an interaction of 'cDC x TIME' (Table 3-2.) The reason for lack of plasticity response at the group level was a high degree of variability of PAS25 response at the individual level. Some patients facilitated to PAS25 and some patients inhibited to PAS25 in the sham condition. In several previous studies of PAS, participants have been specifically chosen on their ability to produce facilitation after PAS25 and "non-responders" or inhibitors to PAS were not included in these studies[116]. When we followed a similar rationale, grouping patients as facilitators or inhibitors, anodal cDC significantly reduced the facilitatory PAS25 response in FDI and ADM (p=0.032, p=0.038 respectively), with a non-significant reduction in APB (p=0.054). There was a weak tendency for cDC to reduce the amount of suppression in patients who showed inhibition of MEPs after PAS25, suggesting that anodal cDC might reduce the magnitude of PAS25 in either direction.

		'cDC' (1,9)		'TIME' (2,18)		'cDC' x 'TIME' (2,18)	
		F-value	*p-value*	*F-value*	*p-value*	*F-value*	*p-value*
RMT (%SI)	**APB**	.207	.660	1.75	.202	2.10	.151
AMT (%SI)	**APB**	.152	.706	.518	.604	1.26	.309
30MEP (mV)	**APB**	.002	.965	.301	.744	1.55	.239
	FDI	.318	.587	1.36	.283	3.32	.059
	ADM	1.24	.294	.154	.859	2.56	.105
***r*RC**	**APB**	.078	.789	.208	.814	.624	.547
	FDI	.072	.794	.235	.793	.444	.648
	ADM	.214	.655	.444	.648	.293	.749
CSP (ms)	**APB**	.294	.601	1.80	.194	.155	.857

Table 3-2 Effect of cDC on all electrophysiological markers.

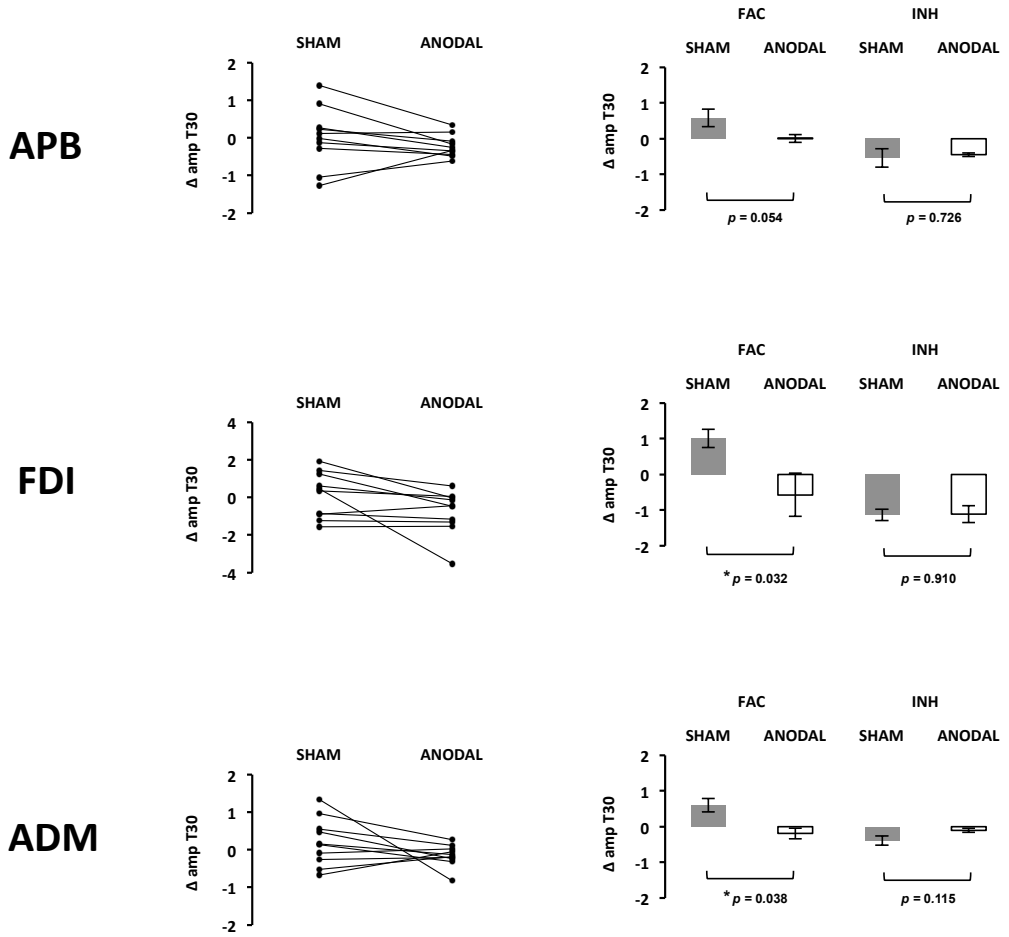


Figure 3-1 Effect on cDC on PAS25 response

For each of the hand muscles tested linked individual PAS25 responses without (sham) and with anodal cDC are shown. Responses are shown as change in the amplitude at T30 (Δ amp T30). To the right, patients are grouped as either facilitators (FAC) of inhibitors (INH) to PAS25 in the sham condition. Overall this data suggests a stabilising effect of cDC on PAS25 response and a significant reduction in the size of PAS25 response in facilitators for the FDI and ADM muscles.

	WCRS			Timed writing (s)			VAS (%)
	Baseline	T30		Baseline	T30		T30
Sham	4.7 ± 0.4	4.7 ± 0.4	No change	74 ± 9.6	73 ± 7.7	$t(14) = 0.35$, $p = .73$	+10 ± 4.8
Anodal	4.7 ± 0.4	4.7 ± 0.4	No change	71 ± 8.3	69 ± 7.9	$t(14) = 0.52$, $p = .61$	+15 ± 5.7

Table 3-3 Clinical ratings after sham and anodal cDC.

See method for description of outcomes. Data are displayed as mean ± standard error of the mean (SEM).

In both stimulation settings there was a moderate subjective improvement in writing which is likely to be a placebo effect (VAS 10 and 15% improvement in sham and anodal cDC respectively). There was no change in the WCRS score or the timed writing assessments in either condition (Table 3-3).

3.4 Discussion

This study has examined the role of cDC as a potential therapeutic tool in WD. Our experimental design incorporated converging evidence from animal and human research. Our study was negative and does not provide evidence that anodal cDC is beneficial for patients after a single session. We discuss the importance of these results alongside recent publications from this currently topical field of dystonia research.

3.4.1 Modulating cerebellar function as a potential treatment for dystonia

We have previously demonstrated that cDC reduces the response to PAS25 in healthy subjects[97]. We had reasoned that since cortical plasticity has been reported to be increased in dystonia, then application of cDC might reduce and normalise the overactive response to PAS. In fact we found that cDC had no effect when all patients were grouped together. At first sight this is a disappointing result, and similar to a recent finding[117]. This group had previously found that PAS25 could be reduced by repetitive TMS over the cerebellum (the continuous theta burst paradigm, cTBS); as in the present study, there was no effect in patients with dystonia.

We believe these negative findings obscure a more important feature. In both studies there was considerable variation in the response to PAS between individuals. In some patients corticospinal excitability following PAS25 was facilitated (i.e. long term potentiation (LTP)-like response) and in some patients it was inhibited (i.e long term depression (LTD)-like response). In fact, there was no net plasticity response at the group level to PAS25 in our present group, and no increase in plasticity compared to control in the work of Meunier et al[118]. We do not think this is due to specific methodological problems since the situation is similar in healthy volunteers: PAS25 produces a facilitatory effect in 30-50%.[119,120] We hypothesise that the response to PAS25 is, as in healthy people, highly variable in patients with dystonia.

Previous studies on healthy participants have circumvented the variability of PAS by preselecting individuals who have a facilitatory response.[121] When we followed the same logic and separated the patients into “responders” who showed facilitation after PAS25 and “non-responders” or inhibitors, we found that cDC reduced facilitation in “responders”, as described previously in healthy individuals. Indeed, there was a weak tendency for cDC to

reduce the amount of suppression in “inhibitors” to PAS25, suggesting that cDC might stabilise the response to PAS25.

Clinically we did not see any behaviourally relevant improvement in measures of WD severity. It is possible that the WD scores we employed were insensitive to clinical changes, however subjective change was also negative and thus we do not think we have missed subtle changes in writing kinematics. In addition the negative electrophysiological data, which motivated our study design, are also against this.

So will the exciting work in animal models of dystonia translate into new therapeutic avenues in humans with dystonia? In rodent models modulating cerebellar function (i.e. cerebellectomy, functional block of output) is sufficient to abolish dystonia. In humans non-invasive stimulation techniques have been unable to achieve this. Any study employing a single session of stimulation is ambitious as one is attempting to undo dystonic processes within the brain, which have presumably been strongly consolidated through many years of symptoms. Clearly cDC and theta burst paradigms are by necessity weaker modulators. Repeated sessions of stimulation (as utilised in the treatment of depression[122]), phasic cDC or more invasive cerebellar stimulation sites are just a few of the potential tools that could be employed in future work. Our view is that cerebellar stimulation with the aim of modulating plasticity responses of the motor cortex in focal hand dystonia is not a useful avenue of research since not all patients have increased plasticity. However further characterisation of pathophysiological changes in dystonia and characterisation of cerebellar dysfunction in humans may well yield the development of new therapeutic options utilising cerebellar modulation, via different underlying mechanisms.

Based on the results of this study cerebellar stimulation may have a role regulating the responsiveness of the motor cortex to plasticity inducing protocols. Aside from dystonia, other important conditions such as brain recovery after stroke would greatly benefit from a non-invasive brain stimulation method that regulates plasticity response. It may be that in other conditions, if exaggerated plasticity is more consistently seen, a therapeutic effect is possible, especially if stimulation is repeated and given alongside targeted physical rehabilitation.

4 Eye blink conditioning and dystonia

4.1 Introduction

Eye blink conditioning (EBC) is a form of Pavlovian conditioning that has been used extensively to study neural structures and mechanisms that underlie learning and memory[30]. The procedure consists of pairing an auditory or visual stimulus (the conditioned stimulus (CS) with an eyeblink-provoking unconditioned stimulus (US)). Naïve organisms thus initially produce a reflexive unconditioned response (UR) that follows US onset. After many CS-US parings an association is formed such that a learned blink or condtiioned respone (CR) occurs and precedes US onset. It was first developed for use in human participants in the 1920s but its presence across species has allowed detailed analysis of its mechanism across experimental levels. EBC is dependent on an intact cerebellar circuitry and afferent and efferent components of the reflex can be beautifully linked to the activity of individual cerebellar cells on a trial to trial basis[30]. More recently it has been used as a marker of cerebellar function in human disease groups. For example rates of conditioning are reduced in cerebellar degeneration[123] and also diseases in which functional deficits of the cerebellum are suspected (such as essential tremor[124]).

DYT1 and DYT6 are typically generalised dystonias with identified genes (TorsinA and THAP1). The case for cerebellar involvement is perhaps particularly strong in DYT1 dystonia due to the ability to investigate the disease using animal models which are increasingly refined in their ability to probe and implicate the cerebellum[125]. In humans, neuroimaging suggests that both DYT1 and DYT6 have reduced integrity of the cerebello-thalomo-cortical tract and metabolic cerebellar abnormalities have been identified in functional imaging studies[66]. Eyeblink conditioning (EBC) is a form of associative learning that has been shown to be critically dependent on the cerebellum in both animal [126], and human studies [127]. Patients with cervical and focal hand dystonia have lower rates of conditioning compared to controls[85], and this is perhaps the most direct evidence in humans that there is cerebellar dysfunction in focal dystonia.

In this study we examined DYT1 and DYT6 dystonia to determine if these patients also demonstrate impairments in EBC or changes in the blink reflex recovery cycle (BRR, a marker of brainstem excitability). These two paradigms provide a unique window into the function of the brainstem and cerebellum in these genetic dystonias.

		Age (yr)	Dur (yr)	Trem	Meds	Burk-Fahn-Marsden motor score									
						Eyes 0-8	Mouth 0-8	S&S 0-16	Neck 0-8	RA 0-16	LA 0-16	RL 0-16	LL 0-16	Trunk 0-16	TOTAL Max 120
DYT1	F	22	13	No	BTX	0	0	0	4	6	6	0	0	0	**16**
DYT1	F	30	19	No	Nil	0	0	0	2	6	4	4	0	0	**16**
DYT1	M	40	11	No	THP	0	0	0	8	0	0	0	0	4	**12**
DYT1	F	43	31	No	THP, CLZ	0	4	8	6	2	4	6	6	6	**42**
DYT1	M	44	7	No	Nil	0	0	1	6	0	0	4	1	9	**21**
DYT1	F	47	39	Yes	THP, CLZ	0	0	0	4	9	4	4	0	1	**22**
DYT1	M	49	35	Yes	Nil	0	0	0	4	12	12	0	0	6	**34**
DYT1	M	50	30	No	BTX, THP	0	6	1	8	12	12	4	4	8	**55**
DYT1	F	67	56	Yes	THP, CLZ	0	0	0	6	12	12	9	9	9	**57**
DYT1	F	72	70	Yes	BTX	0	0	2	0	0	12	4	12	0	**30**
DYT1	M	81	46	Yes	Nil	0	6	0	6	12	8	6	2	4	**44**
DYT6	F	23	14	Yes	THP	0	8	8	6	2	4	6	6	6	**46**
DYT6	F	26	19	No	BTX, THP	0	0	1	6	0	0	4	1	9	**21**
DTY6	F	34	24	No	BTX	0	0	0	4	6	6	0	0	0	**16**
DTY6	F	36	33	No	Nil	0	0	0	8	0	0	0	0	4	**12**
DTY6	M	66	49	No	BTX, THP	0	6	0	6	12	8	6	2	4	**44**

Table 4-1 Patient characteristics

Red indicates severely affected. None of the patients had blepharospasm. Yr: years, Dur: duration of disease at time of testing, Trem: tremor, Meds: medications at time of study, BTX: botulinum toxin injections, THP: trihexyphenidyl, CLZ: clonazepam, S&S: speech and swallowing, RA: right arm, LA: left arm, RL: right leg, LL: left leg, Max: maximum possible total score of Burk-Fahn-Marsden motor score is 120.

4.2 Method

Eleven DYT1 and 5 DYT6 patients were recruited from the National Hospital for Neurology and Neurosurgery, London. Patients were individually age-matched to controls as the ability to acquire eye blink conditioning changes significantly with age[128]. All patients that received botulinum toxin injections were tested at least three months after their last treatment. Clinical details and medications are given in (Table 4.1). The study was approved by the local ethics committee and written informed consent was obtained.

Electrical stimulation (square wave, 200μs) of the supraorbital nerve was applied using chloride disc surface electrodes (cathode: right supraorbital foramen, anode: 2 cm above). Eye blinks were captured by surface EMG electrodes over right and left orbicularis oculi muscles and signal was amplified (gain 2000), bandpass filtered (20Hz–30,000Hz), digitised (5kHz), and stored for offline analysis using Cambridge Electronic Design (CED) 1401 hardware and Signal software (CED).

The EBC paradigm was identical to previous publications[85]. The conditioning stimulus (CS) was a loud (~70dB), 2000Hz, 400ms tone via binaural headphones. The unconditioned stimulus (US) was an electrical stimulus (200μsec, 5 times sensory threshold) to the supraorbital nerve at the termination of the CS which elicited a blink reflex (US). Repeated

pairs of CS and US yielded conditioned blink responses (CRs) occurring before the US (Figure 4-1A). EMG bursts were regarded as CRs if latency was >200ms after onset of CS but before the US. Six blocks of 11 trials (9 x CS-US, 1 x US and 1 x CS) were performed. US-only detects rates of spontaneous blinks and CS-only confirms that CRs are acquired independent of US. The seventh block measured extinction with 11 CS-only trials in which EMG bursts occurring 200–600ms after the CS were considered CRs.

BRR was measured by applying pairs of electrical stimuli at 5 times sensory threshold to the supraorbital nerve at interstimulus intervals (ISI) of 200ms, 300ms, 400ms and 1000ms [129] in a pseudorandomised manner. The bilateral R2 component of the EMG response to the second stimulus is typically suppressed at ISIs of 200ms, 300ms and 400ms,[129]. In subtypes of dystonia the lack of R2 suppression at these intervals is generally regarded as a marker of the increased brainstem excitability [130]. For each trial, EMG data from the non-stimulated left orbicularis oculi were rectified and the area ratio of the first and second R2 responses was calculated following subtraction of the mean prestimulus background activity (R2 *duration*mean background activity*). This was necessary to compensate for the higher levels of resting EMG in patients with dystonia of the face. Mean values were calculated from the 8 trials for each ISI.

Rates of CRs during EBC were assessed using repeated measures ANOVA (rmANOVA) (SPSS for Windows, USA version 21) with "block" as within-subject (block1:6) and "group" as between-subjects factor (dystonia, normal). Extinction rates in subjects who successfully conditioned (defined as >40% CRs in any block) were examined using paired student t-tests comparing the % of CRs in block 6 to % of CRs in the extinction block. BRR was assessed using rmANOVA with "ISI" as within-subject factor (200ms, 300ms, 400ms, 1000ms) and "group" (dystonia, normal) as between-subject factor. Greenhouse-Geisser method was used to correct for non-sphericity and Bonferonni correction was used with multiple comparisons. Otherwise statistical significance was $p<0.05$.

4.3 Results

Patients with DYT1 dystonia (Figure 4-1 B-D) had comparable rates of conditioning to controls: effect of "block" $F(3.16,63.3)=11.62$, $p<.001$; but no "block*group" interaction $F(3.16, 63.3)=1.17$, $p=.33$) or effect of "group" $F(1,20)=.128$, $p=.725$. Seven DYT1 patients and nine controls acquired the CR to a level of 40%. Both patients ($p=.0068$) and controls ($p=.0094$) exhibited extinction.

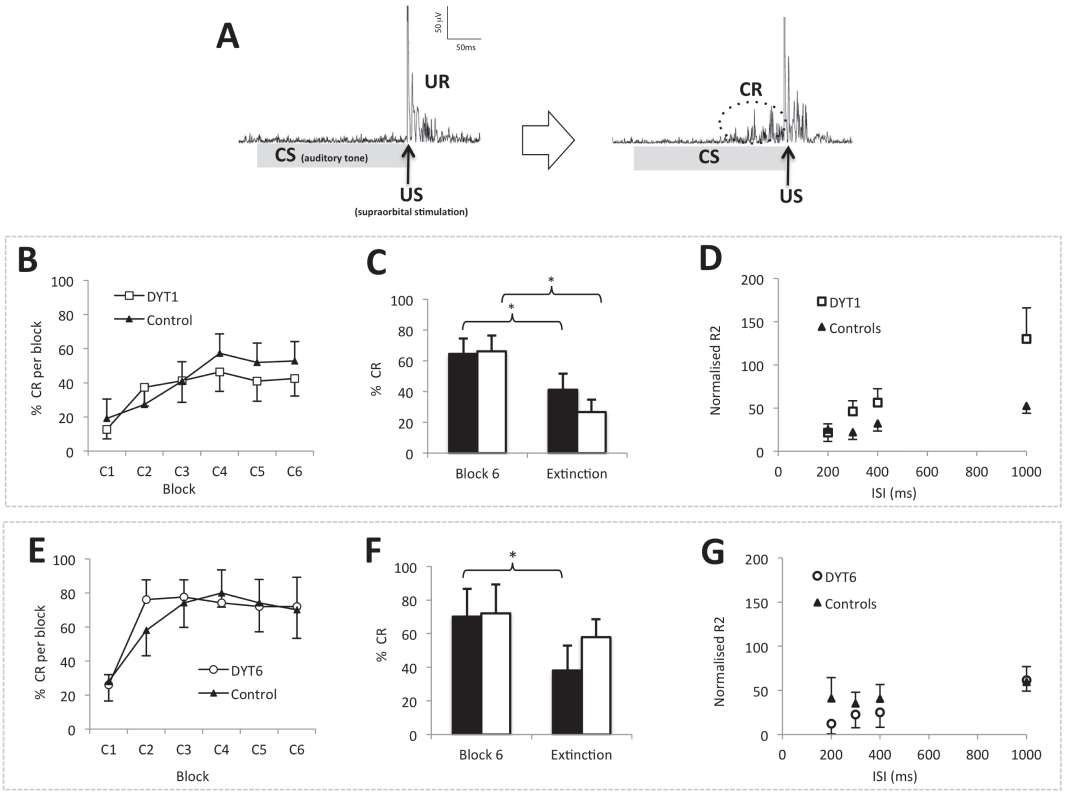


Figure 4-1: Eye blink conditioning and blink reflex recovery cycle in DYT1 and DYT6

A) Rectified EMG traces from orbicularis oculi demonstrating EBC paradigm and responses before and after conditioning has developed. B & E) EBC over six conditioning blocks C & F) Shows extinction rates for dystonia (clear bars) and controls (black bars). D & G) BRR at different ISI. In DYT1 there was a different time profile of inhibition with greater recovery of R2 at later ISI

EBC was also comparable in patients with DYT6 dystonia (Figure 4-1 E-G) and controls: effect of "block" $F(5,40)=15.4$, $p<.001$; but no effect of "block*group" $F(5,40)=.752$, $p=.60$ or "group" $F(1,8)=0.16$, $p=.903$) (Figure 4-1E). The fact that all patients with DYT6 conditioned to high levels, despite the small group numbers, suggests that there is no impairment in acquisition of EBC in this genetic group. All DYT6 patients and controls acquired CR to a level of 40%. Unlike controls ($p=.030$), DYT6 patients failed to show significant extinction ($p=.525$). Although EBC appears to differ between DYT1 and DYT6, this is likely to be due to a greater proportion of younger people in the DYT6 group (80% of DYT6 patients < 40 years old, 18% of DYT1 patients). The ability to acquire EBC is critically dependent on age, [128] and therefore a comparison between the dystonia groups was not performed. A majority of patients in both groups were receiving treatment for dystonia when the study was performed (oral medications or botulinum toxin injections). We propose that EBC is within normal limits in both genetic groups. As none of these treatments should enhance the ability to acquire EBC [131,132], we do not think that the fact that patients were receiving treatment is obscuring a deficit in EBC.

One DYT1 patient, one DYT6 patient and two controls did not complete BRR as they found the paradigm uncomfortable. BRR differed between DYT1 patients and controls: effect of "ISI" $F(1.56,25.0)=12.0$, $p=0.001$, and "ISI*group" $F(1.56,25.0)=3.83$, $p=0.045$, but no effect of "group" ($F(1,16)=2.11$, $p=0.166$)). Post hoc analysis did not show significant differences at an individual ISI. No difference in BRR was seen between DYT6 patients and controls: effect of "ISI" $F(1,41,9.86)=5.15$, $p=0.038$, but not "ISI*group" $F(1.41, 9.85)=.828$, $p=0.425$ or "group" $F(1,7)=.432$, $p=0.532$.

4.4 Discussion

In this study we show that patients with DYT1 and DYT6 have EBC rates comparable to controls. In addition, patients with DYT1 have differences in their BRR to controls, which suggests reduced inhibition within brainstem circuits, and this effect was not observed in DYT6.

EBC is critically dependent on intact olivo-cerebellar function, and is abnormal in patients with focal hand and cervical dystonia [85,133]. The normal EBC seen in DYT1 and DYT6 is thus in contrast to these focal dystonias and suggests that the different forms of isolated dystonia may have different neuroanatomical correlates. It is intriguing to hypothesise what this signifies. Most obviously our data may have their origins in the phenotypical differences associated with subtypes of dystonia. The genetic background of most focal dystonias is still unknown, the disease occurs later in life and there is a greater influence of environment factors. Perhaps the cerebellum takes a greater compensatory role in focal dystonia to counteract the dystonic motor activity and due to competing demands on its net function this impairs the ability of the cerebellar networks to acquire CRs. However, this is unlikely to be the whole story. EBC is normal in patients with secondary dystonia [134] caused by basal ganglia lesions, arguing against a straightforward compensatory role of the cerebellum in alleviating symptoms of dystonia.

Our results are surprising as evidence in support of cerebellar deficits in DYT1 dystonia, in particular, is perhaps stronger than for focal dystonia. The absence of clinical signs of cerebellar dysfunction in patients with isolated dystonia highlights that if the cerebellum is implicated in the pathophysiology it is likely to be a selective impairment of a pertinent feature of motor control. Our patients with genetic dystonia, at least in the circuits essential to EBC, seem to have normal cerebellar function. Furthermore this type of associative learning with its clear dependency on recognising salient sensory inputs within millisecond timing intervals is not impaired.

The BRR in DYT1 patients showed hyperexcitability in line with previous experimental results that have tested the BRR in generalised dystonia, segmental dystonia, focal cervical

dystonia and dystonic hand tremor [85,130,135]. In contrast DYT6 patients did not differ from controls a finding which has previously been observed in focal arm and hand dystonias [85,130]. This was surprising as the DYT6 patients had clinical involvement of cranio-cervical muscles (although none had blepharospasm) and this has previously been thought to be a factor in determining the extent of abnormal brainstem interneuron function[130]. Our findings add further complexity to the need to define a specific electrophysiological abnormality of the BRR and its functional significance in dystonia pathophysiology. The BRR is also disinhibited during voluntary eye musculature contraction in healthy controls, peripheral disorders that evoke facial muscle contractions and other movement disorders[136-138]. It is currently unclear what the differences in BRR profiles across the subtypes of dystonia signify. Recently the BRR has shown surprising ability (100% sensitivity and specificity) to dissociate between tremor subtypes that have been first classified clinically as dystonic tremor or essential tremor [135] and has been proposed as a potential test for dystonic tremor. Our results and those of others, suggest that caution should be taken when proposing a single electrophysiological abnormality as diagnostic of dystonia.

The main limitation of our study is the small number of available subjects with DYT6 dystonia, which reflects the lower prevalence of this genetic dystonia and further multicentre studies are encouraged.

The cerebellum has received increasing attention as an important neuroanatomical structure involved in the pathophysiology of dystonia. However this research is still at an early stage and it remains difficult to obtain direct evidence in humans to specifically implicate the cerebellum in dystonia. Our data suggest that the circuits involved with EBC within the cerebellum maintain normal function in DYT1 and DYT6 dystonia.

5 Millisecond timing in cervical dystonia

Patients with dystonia have elevated temporal discrimination thresholds (TDT: the shortest detectable interval between two stimuli), which are thought to indicate the presence of a basic deficit in sensory processing. Current paradigms may test a range of sensory processes and non-sensory processes. In order to better establish the precise psychophysical deficit in cervical dystonia (CD) I designed two novel tasks specifically explored millisecond timing estimation. If a timing deficit was at the root of elevated temporal discrimination thresholds in dystonia this may be indicative of abnormal cerebellar function.

5.1 Introduction

Dystonia is a movement disorder characterised by abnormal postures due to involuntary muscle contractions. Frequently individuals use alleviating manoeuvres (sensory tricks) to reduce the severity of abnormal muscle activity[139]. The importance of such sensory influences has received much attention experimentally and a range of abnormalities in the sensory domain have been discovered[140-142]. One of the most widely studied perceptual measures is the temporal discrimination threshold (TDT) which has been defined as the shortest interval at which subjects can perceive that there are two stimuli rather than one [143]. Elevated thresholds are present across subtypes of isolated dystonia [144]. Furthermore the finding that TDTs are abnormal in first-degree relatives of those with dystonia has led the suggestion that the TDT represents an endophenotype. Correspondingly there has been much speculation on how mechanisms underpinning abnormal thresholds may inform on the pathogenesis of dystonia [144-146].

Although the TDT assesses temporal discrimination, current paradigms also test extraneous sensory and non-sensory processes (Figure 5-1). The use of an explicitly increasing or decreasing separation between two stimuli can be readily biased by decision strategy unrelated to temporal discrimination ability. Furthermore behavioural and cognitive changes which are increasingly considered part of the dystonic phenotype are likely to define an individual's decision making criterion and carry much influence over threshold values obtained using existing psychophysical methods. Elevated TDTs are seen across a range of other hyperkinetic, hypokinetic and functional (psychogenic) movement disorders (Figure 5-1)[144,147-151]. Disease-specific abnormalities may be hidden within such a composite TDT metric and better quantification of the precise deficit might offer better insight into the pathophysiological mechanisms involved in these distinct diseases.

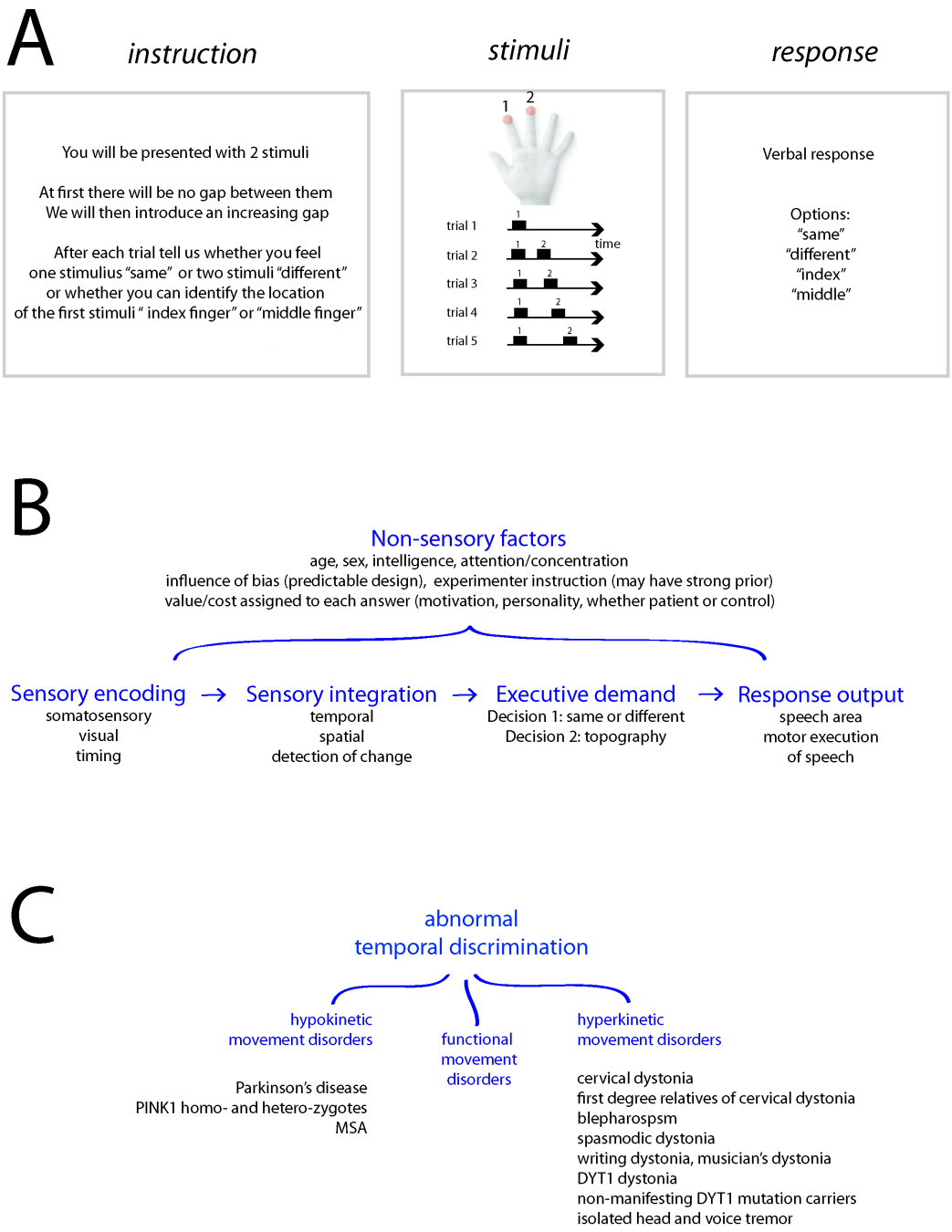


Figure 5-1: Features of commonly used TDT paradigms

A *Example of commonly used ascending staircase methodology. The somatosensory version uses electrical stimulation of index and middle finger. The interval between stimuli is increased systematically until subjects consistently report the location of the first stimulus. The visual version tests the ability to discriminate flashes from light emitting diodes vertically spaced (location defined as upper or lower) in either the right or left visual fields. The TDT is a mean metric of the four combinations (modality, side) each of which are repeated 4 times (median value used) (16 total runs)* [152]*.* ***B*** *Temporal discrimination deconstructed: a mix of cognitive, sensory and motor elements are assessed.* ***C*** *Movement disorders with impaired temporal discrimination using such methodology: Parkinson's disease, PINK1 homozygous and heterozygous subjects, multiple system atrophy* [147-150]*,*

functional/psychogenic movement disorders [149], adult-onset focal isolated dystonias [144], non-manifesting and manifesting DYT1 gene carriers and isolated head and voice tremors [151].

In the present study I applied vigorous psychophysical methodology to examine temporal discrimination more reliably. A randomised and automatic version of the TDT, called *temporal resolution* had basic elements common to currently used TDT methods and removed potentially confounding elements which are not integral to the definition of resolution/acuity (the ability to detect that two stimuli are present rather than one). A second task, *interval discrimination,* examined the ability of subjects to compare the lengths of two consecutive intervals in the millisecond range. This task was designed to test a different aspect of time perception: temporal discrimination, i.e. the ability to discern differences in the lengths of two intervals. To each of these tasks we applied an established mathematical model of decision-making that can disentangle sensorimotor processes from evidence accumulation and decision strategy, each of which could potentially be abnormal in dystonia.

5.2 Background to psychophysical methodology

Superficially, documenting the temporal discrimination thresholds is a simple procedure. It can be defined as the shortest interval at which subjects can perceive that there is a gap between two stimuli. Each trial represents a choice between two options in which the participant must communicate whether they perceived one or two stimuli. During an experiment the interval between two stimuli is varied and the threshold at which they detect this gap is noted. Ascending and descending staircase designs, in which the interval between stimuli is systematically increased or decreased, have shown similar results in many studies in the literature. Why do these protocols need re-evaluation?

When a series of perceptual decisions occur in a fixed predictable order, perceptual reports are subject to strong biases, due to expectations, range effects and anchoring effects [153,154]. These well established effects are mitigated by randomising the order of presentation, making decisions less vulnerable to systematic variations in criterion. For example in the standard staircase method, the threshold is taken as the first of three consecutive trials at which an observer reports that two stimuli were felt (retrospectively identified). Throughout the experiment, over trials, the interval between stimuli gradually increases (Figure 5-1). The subject being tested has some awareness of the protocol design due to the instruction. Such an approach allows prior belief (e.g. based on previous trials) to influence decision behaviour about upcoming trials:

Use of a forced choice randomised design minimises the use such priors, since the order of 1- and 2- stimulus cannot be predicted. It therefore provides a more accurate measure of the quality of sensory information available for that trial, minimising the confound of decision-making criteria:

Stimulus ➔ Judgement ➔ Response

In order to make the correct answer unpredictable, a mixture of both 1- and 2- stimulus trials are needed. Previous studies have used a single 200μs pulse with no change in stimulus strength as catch trials and we had intended to use this as our 1- stimulus trial. However in pilot testing we found that this was easily discernible from a 2- stimulus trial, not due to the absence of a gap, but due to their subjectively weaker *intensity* by virtue of the fact that the quanta of charge delivered is half, and also because the total *duration* of the two pulses delivered during 2 stimuli trials is 400μs i.e. previous paradigms have had a difference of length of 200μs between 1- stimulus and below threshold 2 stimuli trials.

We left the parameters of two-stimuli trials unchanged compared to previous paradigms (200μs pulse width). For 1- stimulus trials, a second stimulator in parallel was therefore configured to deliver an equivalent pulse quality to below threshold 2- stimulus trials. Firstly a longer pulse was used. As a pulse width of 400μs was not possible with available electrical stimulators we used 500μs for 1- stimulus trials; a difference of length of 100μs between 1- stimulus and below threshold 2- stimulus trials. Secondly, at the start of the experiment the intensity of the electrical stimulation was titrated such that 1- stimulus and 2-stimulus separated by 1ms were indistinguishable. Individual plots for each subject are shown in Figure 5-4. At small intervals all subjects now could *not* discern a gap (first data points close to floor of function) and participants subjectively reported that the 1- stimulus trials were perceived as identical to the 1ms interval 2-stimulus trials.

Previous paradigms set stimulation intensity at 2x or 3x the perceptual threshold. In pilot data we found that in certain subjects this resulted in stimulation strength was too painful to continue (it is unlikely that stimulation strength (mAmp) and intensity perception have a linear relationship in all subjects). As we were interested in the timing qualities of stimuli rather than strength we adjusted stimuli to a level that salient but not painful for all subjects.

5.3 Methods

Twenty-two healthy subjects (mean age 56.2 years, 17 females) and 22 subjects with cervical dystonia (mean age 58.2 years, 17 females) were tested. All dystonic subjects had

clinically apparent postural abnormality and were not tremor dominant. They were between the age of 30 and 75 and were receiving treatment with botulinum toxin injections (tested a minimum of 3 months after their last treatment). No subjects had evidence of significant cognitive disease, other major health problems nor sensory problems in the limbs. All subjects completed Raven's Progressive Matrices (maximum/best performance score 12), a non-verbal test of cognitive ability. The Toronto Western Spasmodic Torticollis Rating Score (TWSTRS) was documented (maximum/worst score 85) for all patients. Written informed consent was obtained and the study was approved by the local Ethics Committee.

Both tasks were performed seated and button presses were made using the index finger of their right hand. An answer was required for every trial even if uncertain of the answer and subjects were prompted to guess if they paused longer than 5 seconds (forced choice). Subjects were trained in each task (20 trials, data discarded) prior to the start of each task. The total length of time of the experiment with both tasks was approximately 30 minutes. Experiments were coded in Matlab using the Cogent toolbox.

5.3.1 Temporal Resolution Task

300 consecutive trials were presented in which subjects were asked to respond with a button press to indicate whether they felt one or two stimuli (Figure 5-2). On each trial either 1 stimulus or 2 stimuli (randomised interval range from 1 to 200ms) were presented. Unknown to participants, the proportion of single-stimulus trials was 30% and of double stimuli trials was 70%. The order of single and double trials was randomised within the 300 trials. The index finger of their left hand was stimulated using a ring electrode connected in parallel with two Digitimer electrical stimulators.

5.3.2 Interval Discrimination Task

After a short break (approximately 2 minutes), subjects were presented with 300 consecutive trials in which they were asked to respond with a button press whether the first or second interval was longer (Figure 5-2). One interval was selected from three fixed values (50ms, 100ms and 200ms) the other interval varied within the range from 1ms up to twice the fixed value (100ms, 200ms and 400ms respectively). All stimuli were 2 x 200μs square wave pulses delivered to the left index finger using a single Digitimer stimulator.

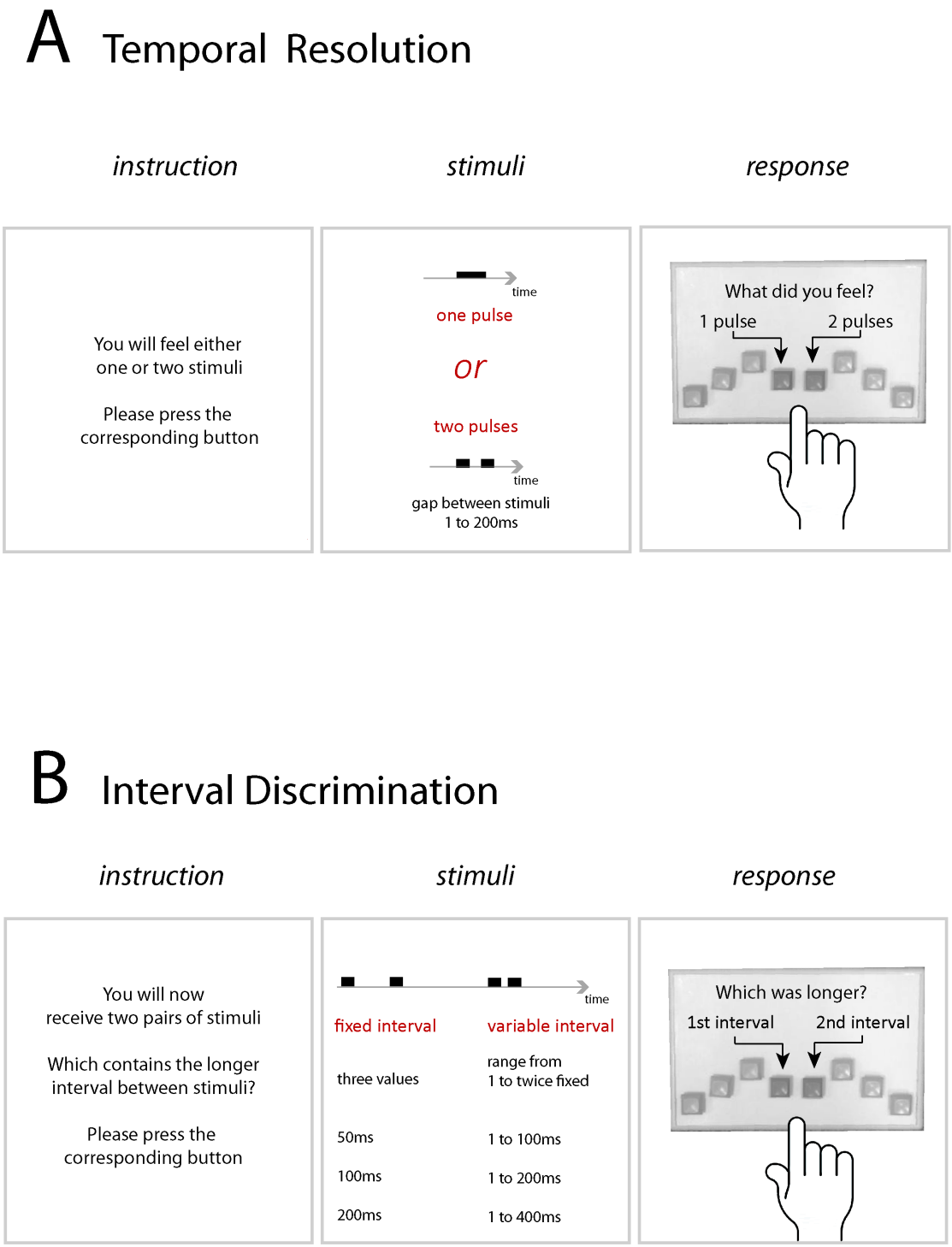


Figure 5-2: Experimental tasks

A Temporal Resolution Task *300 trials in which subjects respond with a button press whether they felt one or two stimuli. Either one pulse or two pulses (with an inter-stimulus range from 1 to 200ms) were presented at each trial.* ***B Interval Discrimination Task*** *300 trials in which subjects respond with a button press to indicate whether the first or second interval was longer. One interval was selected from three fixed values (50ms, 100ms and 200ms) and the other interval varied within the range from 1ms to twice the fixed value (100ms, 200ms and 400ms respectively).*

5.3.3 Psychometric analysis

Data from both tasks were modelled using the cumulative Gaussian (Φ), a mathematical function of sigmoid shape:

$$y = \Phi\left((\log(x) - mu)/sigma\right)/2 + 0.5 \qquad \text{(Equation 5-1)}$$

where y is the proportion of responses on which "two stimuli" were perceived, and x is the interval duration. In the temporal resolution task we used the false positive rate (FP, the proportion of trials where only one stimulus was delivered in which subjects *incorrectly* identified an interval) to define the floor of the function and the logarithm of the interval was used.

$$y = \Phi\left((log(x) - mu)/sigma\right)/2 + 0.5 \times (1 - FP) + FP \qquad \text{(Equation 5-2)}$$

The temporal resolution threshold (mu) was defined as the interval at which the probability of either answer is equal (T_{50}). The slope of the function at T_{50} is equal to the inverse of the standard deviation (1/sigma) of the response distribution. Previous studies of timing in this patient group may only probe responses towards the right of the psychometric function, i.e. when the subject is more certain that there are two stimuli, or when there are a higher proportion of 'two stimuli' responses. Therefore in order to facilitate comparison to other paradigms, we also calculated interval thresholds for T_{75} and T_{98} at which points the probability of reporting "two stimuli" was 0.75 and 0.98 respectively (Figure 5-3A). Akaike's Information Criterion (AIC) was used evaluate the fit of the psychometric model for each subject. This takes into account both the statistical goodness of fit (log-likelihood (LL)) and penalises for an increasing number of parameters (k) estimated to achieve that degree of fit. AIC_{model} was compared to a null model of guessing with lower values indicating the preferred model.

$$AIC_{model} = -2(LL - k) \qquad \text{(Equation 5-3)}$$

For the interval discrimination task the psychometric function was fitted to each subset of data corresponding to each set interval (50ms, 100ms, 200ms) each containing a third of the total trials (Figure 5-6). The point of subjective equivalence (response probability equal for either answer) was used as the threshold value (I_{50}) and the slope was also calculated at this point. In the absence of bias, I_{50} = fixed interval. Slope is a measure of sensitivity: a steep slope reflecting high resolution for the discrimination of interval length. A contrast index was calculated for each trial and was defined as the difference between intervals divided by their total length, (i1=interval one, i2 = interval two):

$$contrast = \frac{i1 - i2}{i1 + i2} \qquad \text{(Equation 5-4)}$$

5.3.4 Drift diffusion model

Response accuracy and reaction times were fitted to the drift diffusion model of evidence integration using the Diffusion Model Analysis Toolbox [155]. This model treats decision time as a period for weighing up information. Mathematically, the distribution of reaction times and errors provides an estimate of the rate of information accumulation (drift rate, v), a decision boundary (a), and non-decision time (nD) (Figure 5-3B). For both tasks, data were divided into seven conditions according to duration of the gap between stimuli (in the temporal resolution task) or contrast (for interval discrimination). These conditions thus varied the strength of evidence favouring a response. The diffusion starting point was fixed halfway between the boundaries, indicating that no information was available about the upcoming stimulus before each trial (randomised nature of both tasks). To confirm that the information accumulation rate explained the difference between conditions, four competing models were evaluated and the model fit was evaluated by total AIC (Table 5-1): (1) Null model. All parameters fixed across conditions. (2) Drift rate free to vary by condition. Decision threshold fixed. (3) Decision threshold free to vary. Drift rate fixed. (4) Both drift rate and decision threshold free across conditions.

	Model detail	Temporal Resolution mean AIC	Interval Discrimination mean AIC
Model 1	Null model. All parameters fixed across conditions	1414	1142
Model 2	Drift rate free. Decision boundary fixed.	909	841
Model 3	Decision boundary free. Drift rate fixed.	1536	3255
Model 4	Both drift rate and decision boundary free across conditions.	1388	3291

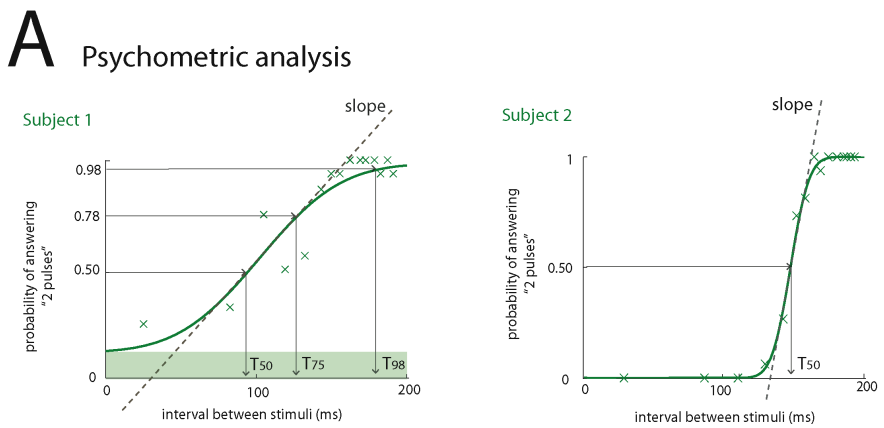
Table 5-1 Drift diffusion model: model fit.

Of the four models assessed, model 2, in which drift rate varied across conditions but decision threshold was fixed was the optimal model for both tasks (as assessed by the Akaike information criteria. Temporal resolution analysis: 77% of subjects were adequately fitted by the model (as defined by AIC values < 3 SD from mean). This excluded four controls and six dystonic subjects from the subsequent analysis. Interval discrimination analysis: 92% of subjects were adequately fitted by the model which excluded three controls from the subsequent analysis.

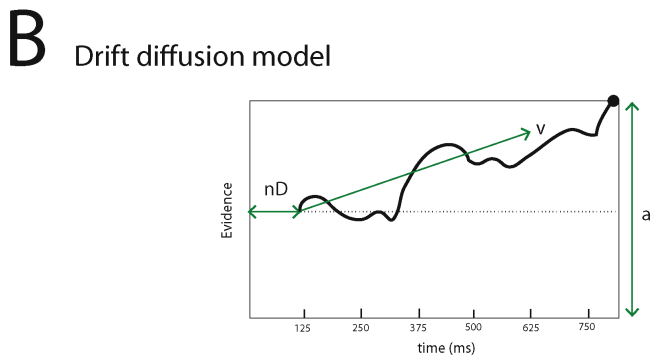
5.3.5 Statistical analysis

To compare distributions between groups, independent t-tests were calculated when the data were normally distributed and the two-tailed Wilcoxon Rank Sum Test for independent samples was used otherwise. Repeated measures analysis of variance across condition was used to compare the drift rate between groups and the interaction of condition by group.

Pearson's correlation was used to estimate covariance of two variables. Data analysis and statistics were performed using Matlab and SPSS.



symbol	parameter	interpretation
T_{50}	0.50 threshold	sensitivity measure at different levels of response certainty
T_{75}	0.75 threshold	
T_{98}	0.98 threshold	
-	0.50 slope	acuity/range of parameter over which decision difficult



	symbol	parameter	interpretation
nondecision	nD	nondecision time	sum of all other processes involved (sensory encoding, motor execution of response)
decision process	v	drift rate	quality of stimulus, amount of input information
	a	decision boundary	criterion setting/speed-accuracy trade off

Figure 5-3: Analysis

A Psychometric analysis. Each graph plots actual data and model from two subjects performing the temporal resolution task. Data were binned into 15 interval ranges and the proportion of trials to which subjects answered "two stimuli" are marked by crosses. Response behaviour was modelled using the psychometric function (solid line). The temporal resolution threshold (T50) was defined as the interval at which subjects answer "2 stimuli" in half of trials. The gradient of the function at T50 was also calculated and is a measure of the range of intervals of decision uncertainty. A steep psychometric function, reflecting more consistent responses, would have a high slope value (slope = Δy/Δx). Subject

1 had a relatively high false positive rate (floor of modelled function, shaded region), T50 is ~95ms and the slope is relatively shallow. Subject 2 had a low false positive rate, their threshold (T50) was greater and the slope is steeper. This demonstrates why both threshold values and slope metrics are complementary when evaluating response behaviour. B Drift diffusion model. The model simultaneously analyses reaction time and accuracy data to allow discrete assessment of the core components of response behaviour. The basic assumption is that in order to make a speeded choice between two options, evidence is accumulated sequentially over time. As soon as sufficient evident toward one option or the other has gathered the process stops and outputs a decision. The accumulation process is governed by two distinct forces, the tendency to drift toward either decision boundary (drift rate, v) and a stochastic component. In this graphical representation of the diffusion process the curved line indicates the amount of evidence for the 'upper' response as it evolves over time. At about 800ms the upper boundary is crossed and the process ends. The distance between the two boundaries, the decision boundary (a) reflects the amount of evidence required before a decision is made. The non-decision (nD) time is the sum of all other processes involved such as the sensory encoding of stimuli and the time required for the motor execution of response.

5.4 Results

There was no significant difference in age (t(42) = -0.598, *p=.838*), sex (17 females in both groups) or intelligence (t(42)=1.84, *p=.076*). The mean TWSTRS score in the patient group was 35.9.

5.4.1 Temporal Resolution

Summary metrics such as the hit rate (proportion of two-stimuli trials correctly identified) and false positive rate (the proportion of one stimulus trials with incorrectly identified as two-stimuli trials) were comparable between groups (Figure 5-4A) The psychometric function fitted the responses of all 44 participants extremely well (Figure 5-4B). The model which simply guesses yields an AIC of 207.9, whereas the mean AIC of the psychometric fit was 101.5 (with no difference in fit values obtained for controls and patients t(42)=-1.32, p=0.191). We had expected subjects with cervical dystonia to demonstrate impaired performance in this task, however we found that performance across groups was remarkably similar (Figure 5-5A). Temporal resolution thresholds (T_{50}, T_{75} and T_{98}) were comparable across groups (Figure 5-4) and there was no significant difference in the slope gradient between controls and cervical dystonia. Therefore despite precise quantification of both isolated thresholds and slope metrics, we found no evidence that temporal resolution, the ability to detect two stimuli, based on accuracy data alone was impaired in cervical dystonia.

Subjects with cervical dystonia were however slower and more variable in their response times (group mean of median reaction time in dystonia 1.07s vs 0.958s in controls, W_m=396, *p=.021*, z=-2.31); and group mean of standard deviation in dystonia 0.133s vs 0.234s in

controls, W_m=389, *p=.013*, z=-2.47). This suggested that despite comparable accuracy data there was a systematic alteration in the timing of responses in dystonic subjects.

In order to obtain more insight into this observation we used the drift diffusion model, which synergistically evaluates accuracy *and* reaction time data in order to quantify separate decision-making components. Given reports that motor function of the limb can be altered in cervical dystonia [156] it was important to show that *non-decision time* was equivalent between groups (median in patients 0.880s vs 0.782s in controls, *ns*). This value is an estimate of the minimum reaction time that would be present even if perceptual discrimination were instantaneous. It is therefore unlikely that increased reaction times observed in dystonia patients were an artefact due to increased time needed to execute the motor response required for the button press. As expected, *drift rate* significantly varied across interval bins (df=3.23, F=12.7, *p=.001),* with lowest drift rates for difficult decisions, close to the perceptual limit. However there was no difference in the drift rates between patient and controls (df=3.23, F=1.60, *p=.191*), indicating that the quality of the information on which decisions were based was not significantly different between groups. In contrast, patients had a markedly elevated *decision boundary* (median in cervical dystonia 0.560 vs 0.293 in controls, W_m=348, *p=.020*, z=2.33). This suggested that dystonic patients had set a different decision criterion, requiring greater evidence before committing to a decision.

A.

	HR	FP	temporal resolution threshold			slope
			T_{50}	T_{75}	T_{98}	
Control	83.4%	2.15%	31.9ms	64.3ms	141ms	38.9
Dystonia	77.4%	1.08%	36.6ms	88.1ms	168ms	31.0
p value	*.197*	*.774*	*.302*	*.302*	*.707*	*.189*

B.

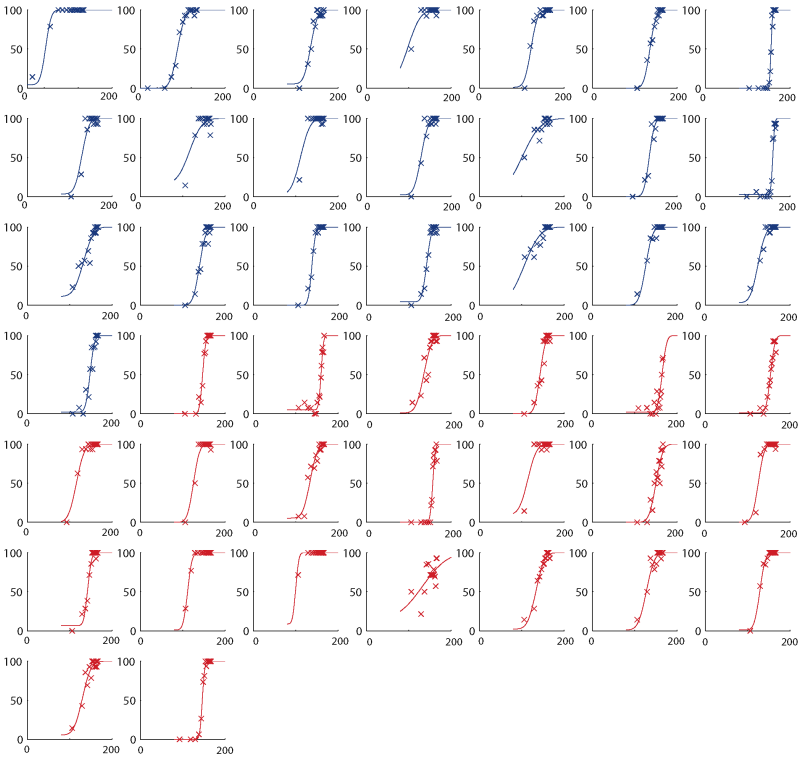


Figure 5-4: Individual plots for temporal resolution task

A Group metrics Hit rate (the percentage of two-stimuli trials in which subjects correctly identified an interval) and false positive rate (FP, the percentage of trials where only one stimulus was delivered in which subjects incorrectly identified an interval) were calculated. Modelled thresholds are given for temporal resolution at $T_{50,}$ T_{75} and T_{98} in order to facilitate comparison to previous studies. The slope at T_{50} has the units: probability of response/ms. p value from the Wilcoxon Rank Sum Test for independent samples given on the lower row of the table for each variable. Subjects with dystonia had a trend for increased thresholds compared to controls at both the T_{75} and T_{98} level, but neither were significantly different. B Individual data Each graph plots actual data and model from an individual subject (n = 44) performing the temporal resolution task. Data were binned into 15 interval ranges (log(interval(ms)), x-axis) and the proportion of trials to which subjects answered "two stimuli" (y-axis) are marked by crosses. Response behaviour was modelled using the psychometric function (solid line). Controls are shown in blue, subjects with cervical dystonia in red.

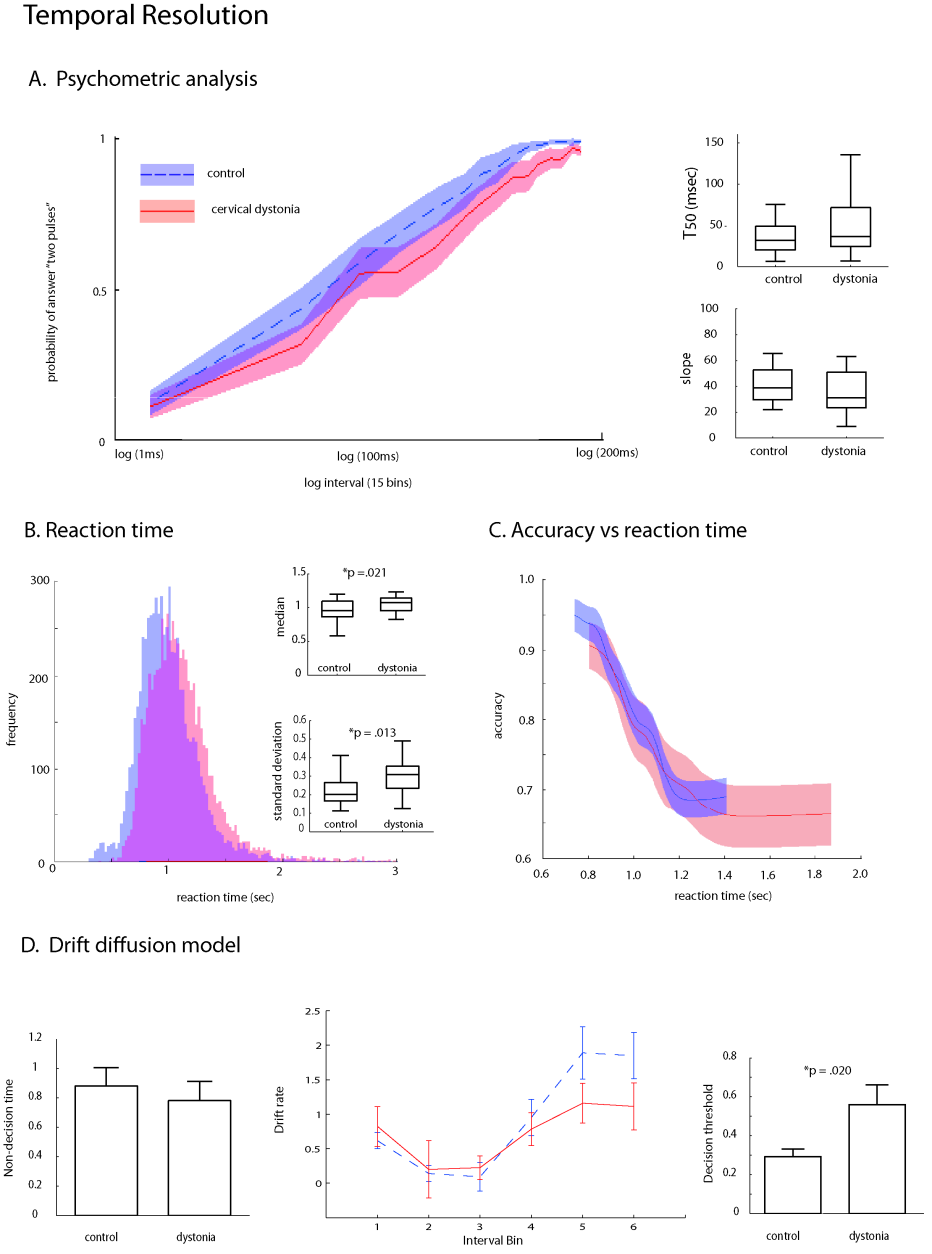


Figure 5-5: Group analysis of temporal resolution task

A Psychometric analysis. A line plot of the probability of answer "two pulses" (y-axis) and log(inter-stimulus interval) (x-axis). Mean control (blue, dotted line) and dystonia (red, solid line) with shaded standard error. There was little difference in response behaviour across the range of intervals tested. The temporal resolution threshold (T_{50}) and slope were no different between groups (midline of box is the median of the data, box spans the 25th to 75th percentile). In addition there was no difference across groups for temporal resolution thresholds reflecting higher response certainty (T_{75}, T_{98}, supplementary Fig 1A) B Reaction time histograms of all trials (200bins) revealed systematic differences in the distribution of reaction times. Both mean median reaction time and mean standard deviation of variance were elevated in the dystonic group. C. Plotting accuracy against reaction time (10 bins) revealed a difference in the manner in which dystonic subjects responded. In the dystonic group the increase in reaction times was most marked for difficult decisions. D Drift Diffusion Model

Evaluation of both response accuracy and reaction time data by the drift diffusion model revealed that the non-decision time was no different between group (bar plot, error bars display standard error). Drift rate representing the accumulation of evidence per unit of time, a marker of the quality of sensory information, significantly varied across interval bins. As 30% of trials comprised the 0ms bin there are six conditions in the model output (bin centres 0ms, 13ms, 44ms, 85ms, 122ms, 158ms). Difficult decisions, close to the perceptual limit, had low drift rate (bins 2 and 3), whereas drift rates further from this point had higher drift rates. The lack of significant difference between groups suggests that there is no significant difference in the quality of sensory information reaching the decision process in cervical dystonia. Decision threshold was increased in cervical dystonia suggesting that patients required greater evidence before a decision was made.

5.4.2 Interval Discrimination

The second task evaluated the ability to discriminate the length of intervals between successive pairs of stimuli. Subjects reported that this task was more difficult than the temporal resolution task, with one control and two dystonic subjects being unable to complete the task (n=41). The psychometric function was fitted for each of the fixed intervals (50ms, 100ms or 200ms, Figure 5-6). No clear group difference in response accuracy was observed, with comparable I_{50} and slope metrics at each fixed interval (Figure 5-6B). Response behaviour using contrast index to combine trials was thus similar across groups (Figure 5-7A).

Compared to controls, subjects with cervical dystonia showed a trend to longer responding for the task but this was not significantly different between groups in terms of mean of median (dystonia 2.42s vs 2.31s in controls, W_m=492, *p=.061*, z=1.87) or variability (mean of standard deviation in dystonia 0.399s vs 0.469s in controls, W_m=484, *p=.097*, z=1.65) (Figure 5-7B). Similar to the temporal resolution threshold, it was decisions around the perceptual threshold (more difficult decisions with lower accuracy) which had the most pronounced increase in reaction time in dystonia (Figure 5-7C).

Modeling data from the interval discrimination task using the drift diffusion model again found no difference in the non-decision time between groups ($W_{m=}$.366, *p=.672,* z=0.424). Diffusion rates were lower than in the temporal resolution task, in keeping with this task being more difficult due to decreased quality of sensory information available. As expected, drift rate approximated zero when there was no contrast between the two intervals and increased with contrast magnitude (Figure 5-7D, df=2.78,F=13.3, p<0.001). Again there were no group differences (interaction of group and drift rate df=2.78, F=1.05, p=.397) suggesting that the quality of sensory information available for the task was equal in both groups. In this task, the decision boundary was not significantly different (dystonia a=0.637 vs a=0.535 in controls, Wm=316, p=.313 z = -1.01).

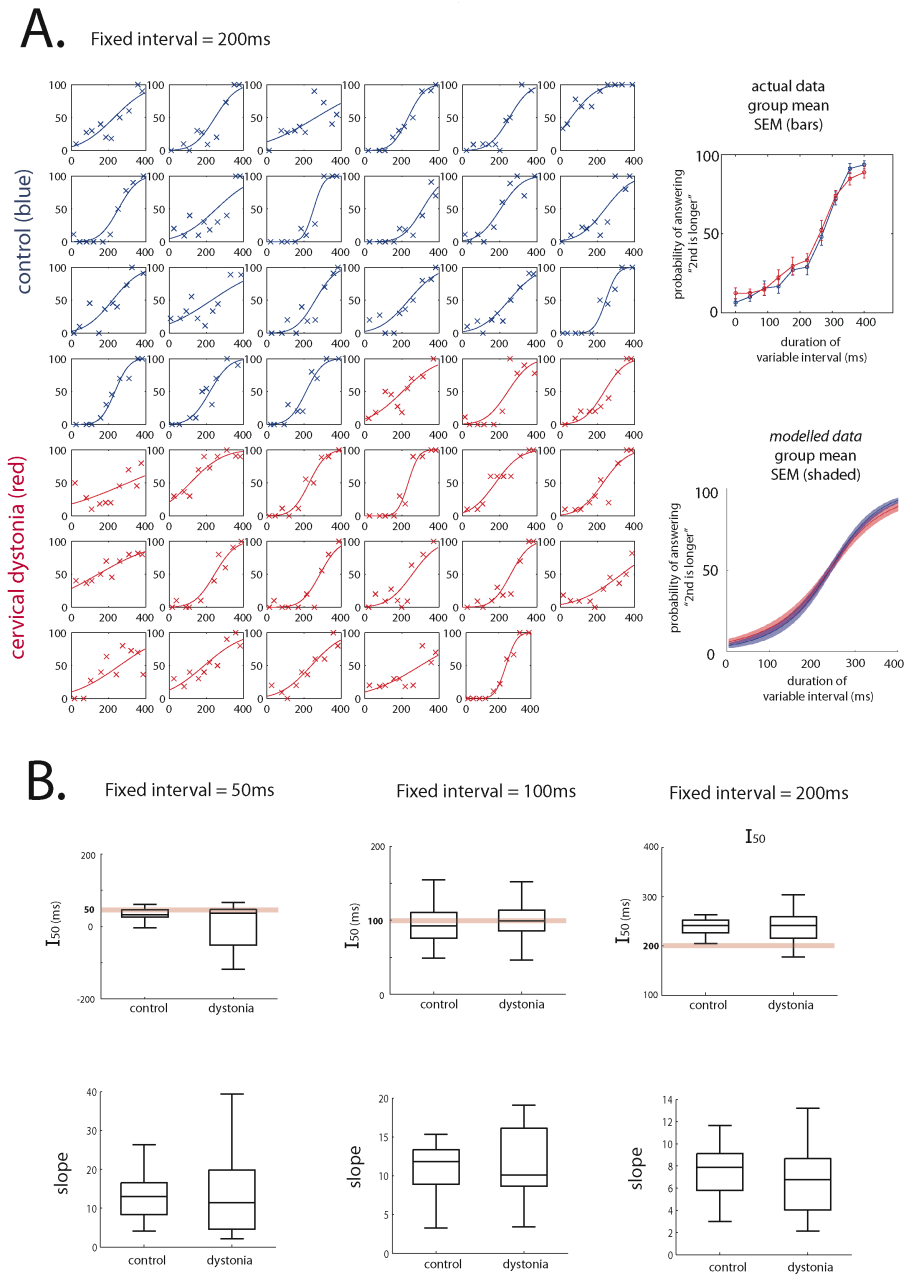


Figure 5-6: Individual plots for interval discrimination task and analysis by interval

Data subdivided by the length of the fixed interval (50ms, 100ms, or 200ms). Each dataset contained approximately 100 trials. Mean accuracy increased as the length of the fixed interval increased (66.5%, 72.9%, 75.4%) reflecting greatest difficulty when the fixed interval was 50ms. Four individuals had no discriminatory ability for when the fixed interval was 50ms (two control, two dystonic) and one control had no discriminatory ability when the fixed interval was 100ms. Data for these subjects were excluded from subsequent analysis. Data from all 41 subjects when the fixed interval was 200ms are shown in A y-axis probability of response "second interval longer", x-axis length of second interval). Crosses are patient data and the solid line is the modelled psychometric function. Group means are shown to the right of the individual data plots. B Boxplots showing no significant difference in I_{50} or slope for any of the three fixed interval values respectively ($p>0.05$ for all rank sum comparisons).

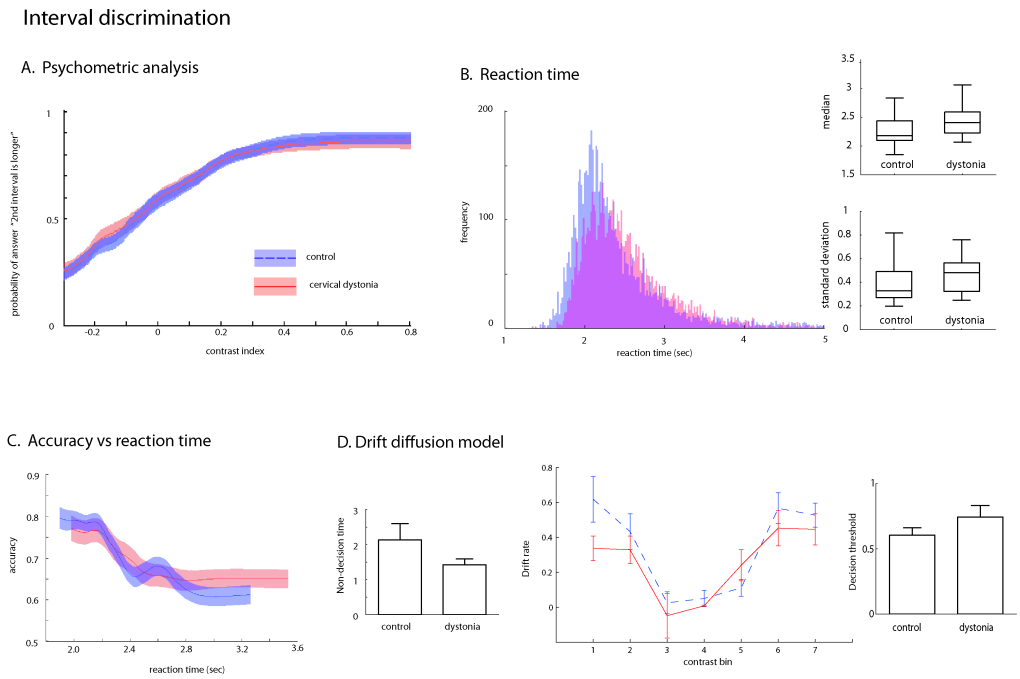


Figure 5-7: Group analysis of interval discrimination using contrast index

A Psychometric analysis Contrast index (equation 4) was used to plot all data. Performance behaviour was similar across the two groups. A negative contrast indicates that the 1st interval was longer than the 2nd interval. As expected response rate approximates 50% when the contrast is zero (no difference between intervals, subjects guessing) B Reaction Time As in the temporal resolution task the reaction time was elevated in the dystonic group but the effect was not significant and variance was comparable. C Accuracy vs reaction time with data divided into 10 bins. Drift Diffusion Model The non-decision time was no different between groups. The drift rate varied significantly with contrast with lowest quality of input sensory information when the difference between intervals was minimal. Bin centres of contrast were -0.288, -0.196, -0.098, 0.037, 0.239, 0.493, 0.813. Drift rate was not significantly different between groups. The decision boundary in the dystonic group was not significantly increased. These results support the hypothesis that another form of timing estimation in the millisecond range is intact in dystonia.

5.4.3 Relationship between tasks

Across individuals the slope in the temporal resolution task correlated strongly with the slopes in the interval discrimination task, as such both tasks appear to sensitively test sensory processing ability in the millisecond range (Figure 5-8).

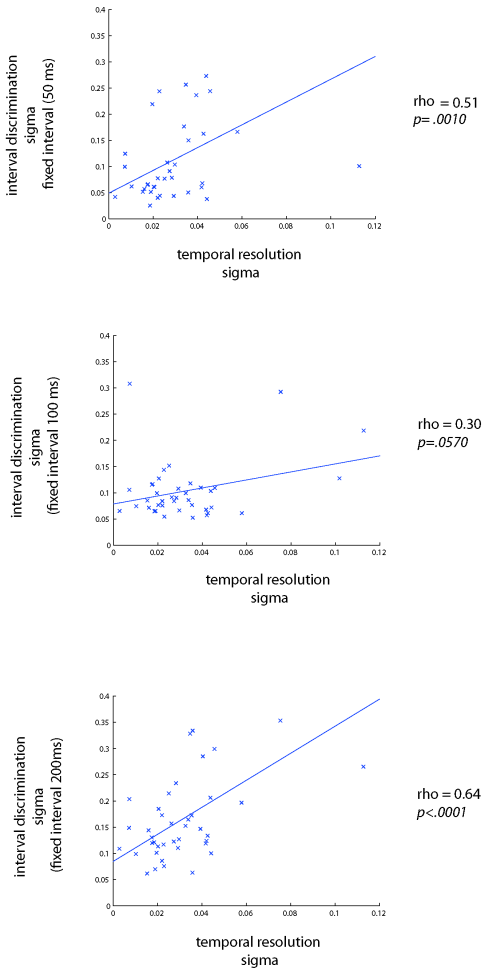


Figure 5-8 Sensitivity of tasks

The standard deviation (sigma) of the psychometric function significantly correlated across tasks. A small value signifies high resolution such that there was only a small range of intervals or contrast of interval through which there was response uncertainty.

5.5 Discussion

Here we present two tasks designed to quantify temporal processing in dystonia. The first task was similar to existing temporal discrimination threshold paradigms, but was randomised and potentially confounding elements were removed. We found no significant difference between patients and controls in accuracy in discriminating single from double stimuli, although patients showed longer reaction times. Analysis of psychometric functions showed no differences between groups but combining reaction time and accuracy data into a decision-making model demonstrated that patients had a higher criterion for information (decision boundary) before responding. A further task investigated the ability to distinguish intervals presented in pairs, and again found patients to be no worse at this task, other than

a trend to slower reaction times. The findings cast doubt on the hypothesis that dystonia is characterised by a specific disorder of temporal processing.

This study used two tasks. The first temporal resolution task was a randomised and automated version of commonly used TDT protocols with other confounding features removed. Our task had no spatial element (two stimuli were not delivered at distinct locations), tested only the somatosensory modality, stimuli order was randomised, single stimulus trials were true catch trials (not recognisable by being of weaker intensity) and response options were binary (other tasks have up to four possible response options which recruits more complex decision making). The second task required comparison of two consecutive interval lengths, and was therefore a test of temporal discrimination. Furthermore for the first time we recorded both accuracy and reaction time since modeling these data in synergy allows assessment of previously unexplored components of the decision-making process.

Testing these tasks revealed no evidence of deficits in temporal discrimination in cervical dystonia. In the *temporal resolution task* patients were equally able to classify one- and two-stimulus. Furthermore the ability to compare the length of two consecutive intervals, *interval discrimination*, was comparable between groups.

Patients were however slower in their responses and demonstrated greater intra-subject variability in response time in the temporal resolution task. We therefore modelled the data using the drift diffusion model which evaluates accuracy *and* reaction time data in order to quantify separate decision-making components. The model confirmed our psychometric results with equivalent drift rates between groups (no difference in the quality of sensory information upon which decisions were based). In the temporal resolution task the decision boundary, i.e. the level of evidence required before a decision is made, was the key difference between groups. As such, in a task with the same components as commonly used TDT tasks, dystonic subjects set a more conservative decision-making strategy (despite the forced choice and randomised design).

Interestingly this may offer a tentative link to psychological research which has shown an increased prevalence of anxiety and depression is likely in cervical dystonia (over 50% in some studies [157]). Anxiety is known to cause shifts in decision boundaries similar to those modelled in the current task [158]. Furthermore such an increase in decision boundary may partially explain elevated thresholds obtained using an ascending staircase design. An increased decision boundary, translates into a bias for subjects to wait before a greater amount of sensory evidence is available before reporting a change in stimuli. Doubt about whether two stimuli were presented on trial *n* will tend to favour postponing the decision to trial *n+1*. These effects are seen irrespective of the quality of sensory signal.

In addition, it is also important to consider differences between our paradigm and traditional methods. For example we delivered stimuli at a single site; it is possible that the spatial integration required to define two stimuli trials delivered at different sites is the core problem in cervical dystonia (any spatial computation is inherently more complex in cervical dystonia due to abnormal head and neck position). Another interesting alternative hypothesis is that threshold abnormalities observed with ordered staircase paradigms are actually testing the ability of subjects to detect a *change* in stimuli rather than temporal discrimination. In line with this argument we have recently shown that mismatch negativity, an EEG event calculated by subtracting the potential produced by a standard repeated stimulus from that produced by a rare 'oddball' stimulus, correlated with TDT obtained by staircase methodology in cervical dystonia. Higher thresholds on the TDT were associated with smaller mismatch negativity thresholds, both suggesting that the saliency of change was reduced [159].

Understanding the neurobiological significance of the documented sensory deficits observed in dystonia is complex. Abnormalities in the detection of stimuli relating to timing, spatial representations, pain, thermal qualities, kinaesthesia have all been documented in the literature [141]. This hints that there may be a common mechanism central to how subjects with dystonia perceive and report sensory phenomena at the root of all of these deficits however the nature of this mechanism remains poorly defined. In this specific task we have shown a change in a core decision-making parameter but it remains to be established whether a more fundamental component of sensory processing is at the root of other sensory deficits.

We have attempted to test as purely as possible perceptual sensitivity for millisecond timing mechanisms and assess the contribution of decision-making components. However the detailed characterisation of psychophysical performance requires careful interpretation, and our results need validation with further studies.

It is relatively recently that the sensory aspects of movement disorders have been championed and their importance in pathogenesis debated. Abnormalities in various domains of sensory processing have been documented in almost all movement disorders yet we are still far from defining how such abnormalities interact to cause the distinct movement disorders. We hope that the application of novel methods and analysis, such as those detailed in this study, will provide better tools to identify disease specific abnormalities in the sensory domain with ensuing insight into the pathophysiology of dystonia and other movement disorders.

6 Adaptation in cervical dystonia

This is the first of two chapters in which adaptation is examined in two subtypes of dystonia. In this study, 20 subjects with cervical dystonia and an equal number of aged matched controls are tested for their ability to adapt to both visuomotor (distorting visual feedback by 30°) and forcefield (applying a velocity-dependent force) perturbations.

6.1 Introduction

In the motor control literature the archetypal cerebellar-dependent paradigm is adaptation. This paradigm requires subjects to adapt their performance of a task (such as reaching to hit a target) after an environmental perturbation (such as distortion of visual feedback) introduces a movement error. The sensory prediction error (how the actual sensory movement outcome differed from the predicted sensory movement outcome) is used to update subsequent motor performance, with this type of learning being strongly dependent on the cerebellum[25]. The cerebellum is thought to be crucial for the formation of forward models, which predict the sensory consequences of a motor command and drive adaptation[26] and is impaired in patients with cerebellar lesions[28,160,161]. Thus, if the ability to adaptation was reduced in dystonia, this could provide a valuable model of the how the cerebellum contributes to the pathophysiology of dystonia.

We have examined the ability to adapt as a marker of cerebellar function in 20 subjects with isolated cervical dystonia and an equal number of aged matched controls. A purpose built robotic arm enabled detailed kinematic analysis of arm movements and we have tested adaptation to both visuomotor and forcefield perturbations for which visual and proprioceptive afferent feedback dominate respectively[162,163]. Our hypothesis was that cerebellar abnormalities observed in dystonia research would translate into deficits of cerebellar adaptation. We also examined the relationship between adaptation and dystonic head tremor as many primary tremor models implicate the cerebello-thalamo-cortical network which is specifically tested by this motor paradigm[164].

6.2 Methods

6.2.1 Subjects

Twenty patients with cervical dystonia were recruited from the National Hospital for Neurology and Neurosurgery, London. Patients were tested at least 3 months after their last botulinum toxin treatment and none were taking oral medications for dystonia. Twenty age-

matched controls were also recruited. Subjects did not have any additional neurological or musculoskeletal problems of the arm or significant cognitive impairment. Written informed consent was obtained from all participants. The study had been approved by the local ethics committee.

Age	Symptomatic head tremor?	TWSTRS		Tremor	
		Severity Subscore	Total	Frequency (Hz)	Power
56	Yes	6	38	NA	NA
76	Yes	2	12	NA	NA
68	Yes	24	44	5.8	85.68
53	No	18	27	5.6	2.11
61	No	15	23	6.6	0.08
75	Yes	2	11	3.1	17.78
63	No	2	14	4.8	0.08
39	No	13	16	6.7	2.40
40	No	19	40	4.3	0.08
40	No	20	43	4.9	0.11
61	Yes	16	44	5.5	14.37
66	No	8	16	7.1	1.07
69	Yes	17	35	3.5	1.02
71	Yes	18	61	3.7	0.39
80	Yes	9	44	3.8	2.83
57	Yes	20	24	4.1	2.14
51	Yes	24	56	7.5	2.48
67	Yes	16	21	3.5	7.20
53	Yes	11	27	4.9	5.59

Table 6-1 Patient characteristics

The severity subscore of the TWSTRS is out of 35. The total TWSTRS which also incorporates disability and pain subscores is out of a total of 87.

6.2.2 Clinical Assessment

Severity of cervical dystonia was examined using the Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS). Head tremor was objectively captured by tri-axial accelerometry prior to the adaptation task with a commodity mobile communication device (HTC Desire) at a sampling frequency of 100Hz and analysed off-line. The device was strapped to the head

below the occipital protuberance. Tremor recordings were made for 30 seconds. Data were analysed with Spike software (CED electronics, version 2). The accelerometry axis with the greatest overall amplitude was used for subsequent analysis. A high pass Butterworth filter (corner 2) was applied and then a Fourier transform of the signal was derived. The dominant frequency was determined by the peak of the frequency spectrum. Total power of the spectra between 1 and 30 Hz was used as a marker of tremor severity.

6.2.3 Robotic apparatus and task

Participants were seated with their forehead supported on a headrest. Their semipronated right hand gripped a manipulandum underneath a horizontally suspended mirror. The mirror prevented direct vision of the hand and arm and showed a reflection of a computer monitor mounted above. The visual display comprised of a central 30 mm square which indicated the starting position, a circular cursor (5mm diameter) representing the position of the manipulandum and a 10mm square target at one of 4 radially arranged positions (45°, 135°, 225° or 315°), 80mm from the starting position. The start of the trial was indicated by the appearance of the target. Subjects were instructed to 'shoot' through the target with a smooth arm movement as this type of movement is thought to rely on feed-forward control; in this type of movement angular error at the start of movement is similar to the angular error at the end of movement suggesting that online feedback processes do not pay a major role in this task [24,27]. The cursor was visible throughout the trial. If movement duration was greater than 300ms the target changed from white to blue at the end of the trial indicating that the movement was too slow. After completion of the outward movement participants were asked to relax and allow the robotic arm to return the arm to the central starting position. Once the cursor was re-centred the next target would appear.

Participants familiarised themselves with the basic task by performing 25 trials during which verbal feedback was given to further explain the desired movement (data not analysed). Each participant then completed 5 experimental conditions in which baseline performance was assessed and then subjects were examined for their ability to adapt and washout both visuomotor and forcefield perturbations (Figure 6-1). The visuomotor condition consisted of a distortion of visual feedback by 30° in the clockwise (positive) or anticlockwise (negative) direction. The forcefield condition consisted of a rightward (positive) or leftward (negative) velocity dependent force applied to the robotic arm during movement (3N/(m/s)). The type of adaptation perturbation was counterbalanced such that if the first perturbation was positive visuomotor the second perturbation was negative forcefield (giving four possible order combinations). The total time of the experiment was approximately 45 minutes.

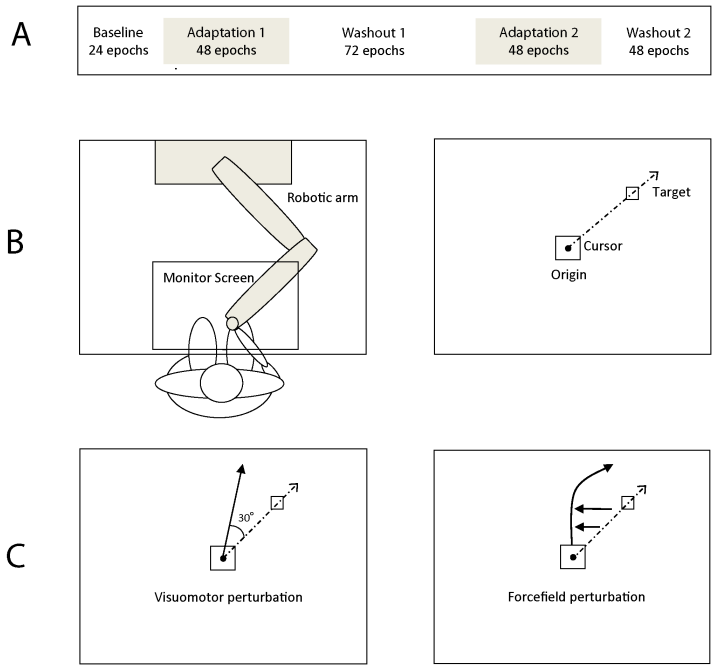


Figure 6-1 Overview of experimental design.

Each epoch consisted of 4 trials. 1B Robotic apparatus and baseline task. Subjects were seated so they looked down at a monitor screen and held the handle of the robot in their right hand. Vision of the handle was blocked by the monitor screen. The position of the handle was visualised as a black cursor on the screen. Participants were instructed to move the cursor to the centre square (starting position). Upon subsequent appearance of the small square (target) in either one of the four corners, subjects were asked to 'shoot' through it. 1C Schematic drawing of the perturbation conditions. The visuomotor condition consisted of a distortion of visual feedback by 30° in the clockwise (positive) or anticlockwise (negative) direction. The forcefield condition consisted of a rightward (positive) or leftward (negative) velocity dependent force applied to the robotic arm during movement (3N/(m/s)).

6.2.4 Kinematic analysis

Hand position was sampled at a rate of 200Hz. The outcome measures were angular error, movement duration and reaction time. Angular error was defined as the angular deviation from the ideal trajectory at the target perimeter. The start time (t_1) of movement was defined as the time point at which 10% of maximal velocity of that trial was reached. This avoided wrongly identifying small corrective movements of the cursor that were not the start of the shooting movement. The end of movement was defined as the time at which the target perimeter was first breached by subject movement (t_2). Movement duration was the difference between these two values (t_2-t_1). Reaction time was calculated as the difference between the time of target presentation (t_0) and the start of movement ($t_1 - t_0$). Trials that had an angular error > ±45 degrees, a movement duration < 200ms or > 800ms, or a reaction

time < 200 or > 600, were excluded (in cervical dystonia 15.7% of trials, in controls 14.3%). Epochs of all kinematic variables were created by taking an average value across four consecutive trials.

The primary outcome, angular error, of the four conditions (visuomotor adaptation, visuomotor washout, forcefield adaptation, forcefield washout) was modelled using:

$$Y = a + bexp^{(-cx)} \qquad \text{Equation 6-1}$$

where ***Y*** represents the predicted angular error, ***a*** is an estimate of the plateau of the learning curve, ***b*** is an estimate of the maximal initial error (the y-intercept), ***c*** estimates the learning index for each condition and ***x*** is the epoch. The learning index is the percentage reduction in error for each epoch and thus can be used as a measure of the rate of adaptation and the rate of washout of perturbations. The adjusted R^2 value was calculated to analyse goodness of fit of the model. If R^2 was less than 0.4 (i.e. explained less than 40% of variation) then the individual's data for that perturbation were excluded from further group analysis (13% excluded).

6.2.5 Statistical analysis

SPSS (IBM SPSS Statistics, v21), Excel (Microsoft Excel for Mac 2011, v14.3.7) and Matlab (R2011b) were used for data analysis and all data are given as mean ± standard error of the mean (SEM). G*Power 3 [165] was used for the power calculation. Learning indices were compared using t-tests with Bonferonni correction for the four conditions (level of significance after correction .05/4). Reaction time and movement duration were compared between cervical dystonia and controls during the fast learning for each condition using analysis outlined in previous studies[24]. For each subject a mean value was calculated during the initial rapid rate of learning such that for the baseline block (total of 24 epochs), epochs 2-6 were averaged and for the adaptation and washout conditions (total of 48 epochs), epochs 2-11 were averaged[166]. Repeated measures analysis of variance (rmANOVA) were used to compare mean reaction time with the factors GROUP (control, dystonia) and CONDITION (baseline, visuomotor adaptation, visuomotor washout, forcefield adaptation, forcefield washout). This analysis was repeated for movement duration. The severity of cervical dystonia as defined by the TWSTRS (both severity subscore and total) and learning index were correlated (Pearson's correlation coefficient (r) and the *p* value are given). To examine for a potential relationship between tremor and adaptation subjects were grouped into clinically apparent tremor and no tremor and t-tests were performed to compare the learning index of the two groups for the adaptation and washout conditions. For patients with clinically apparent tremor, total power as an estimate of severity, was correlated to the learning index for each adaptation/washout condition. Log transformation of total power allowed the subsequent Pearson's correlation.

6.3 Results

6.3.1 Summary

Rates of adaptation (learning) in cervical dystonia were identical to healthy controls in both visuomotor and forcefield tasks. Furthermore, the ability to adapt was not clearly related to clinical features of dystonic head tremor.

6.3.2 Adaptation

All subjects completed the experiments. Mean age and variability were matched between groups (control mean 56.0 years (± 2.46), patient mean 60.3 years (± 2.80), $t(36) = 1.15$, $p=.255$). One patient and one control were excluded from all further analysis due to consistently low movement durations (necessary due to velocity dependent forcefield). In addition tremor data were not available (NA) for two patients due to a technical failure.

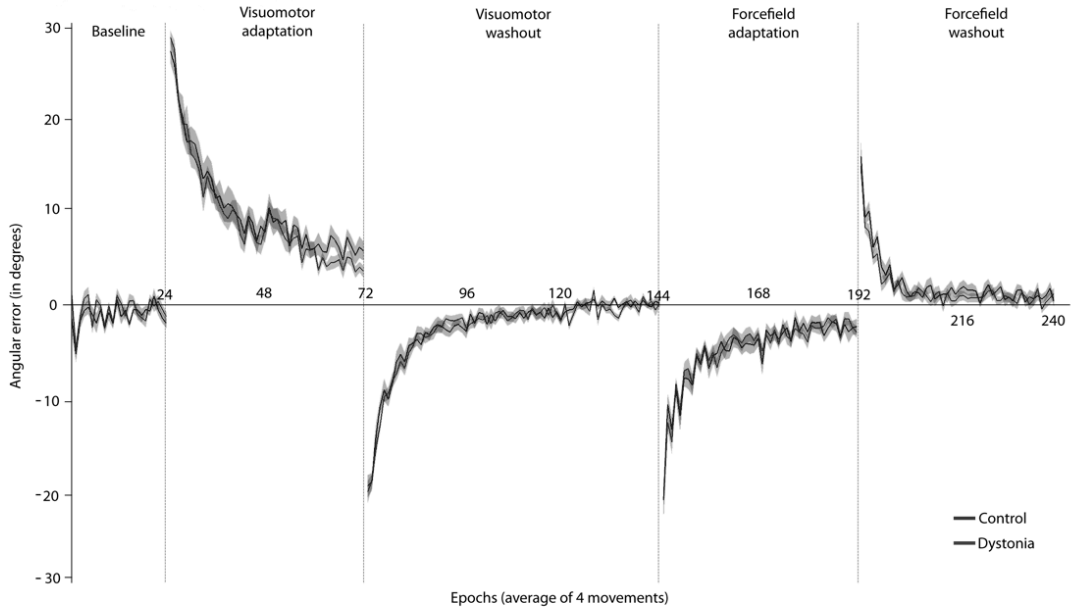


Figure 6-2 Angular error by group

All data sorted into the same order for figure (experimental design consisted of four possible order combinations.) Control data and dystonia data do not demonstrate any differences. The solid line indicates the mean and the shaded regions the standard error.

In Figure 6-2 the angular error for the five conditions is shown for controls and subjects with cervical dystonia. Visually, rates of learning were very similar between groups. To compare rates of learning, an exponential model was applied to each participant's data for the 4 adaptation conditions. Figure 6-3 shows angular error (epochs of 4) of an individual subject in grey and the generated model in red. It can be seen that the model accurately captures

the slope of the curve in each condition which was the main parameter of interest (learning). To be included in analysis, the models adjusted R^2 had to exceed 0.4. The number of exclusions is indicated in Table 6-2. Crucially, out of 152 models 87% reached this criterion. The mean R^2 of the included models were not significantly different between groups: visuomotor adaptation $t(22.7)=-0.770$, $p=0.449$, visuomotor washout $t(33.7)=-0.525$, $p=0.603$, forcefield adaptation: $t(32)=0.413$, $p=0.683$, forcefield washout $t(25)=0.187$, $p=0.853$.

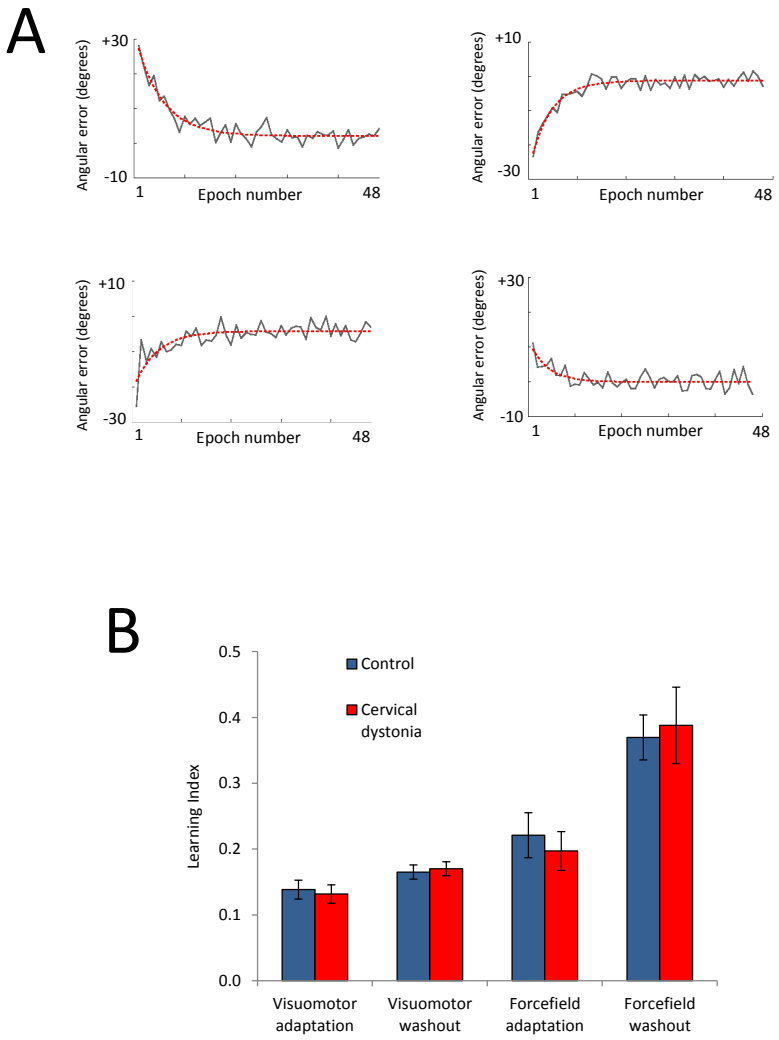


Figure 6-3 Learning outcomes

A The performance of the model for an individual patient in across the 4 experimental conditions. B Bar plot of the mean learning index for control and cervical dystonia with the standard error of the mean indicated by the error bars.

The primary outcome, rate of adaptation and/or washout (mean learning indices) were not statistically different in any of the four conditions: visuomotor adaptation t(33)=-0.396, $p=0.695$, visuomotor washout t(34)=0.287, $p=0.776$, forcefield adaptation t(32)=-0.553,

p=0.584 and forcefield washout $t(25)$=0.254, p=0.801. The profile was also remarkably similar in that for both groups (Figure 6-3): visuomotor adaptation was slower than forcefield adaptation as evidenced by smaller learning indices, washout of the visuomotor perturbations had a rate comparable to visuomotor adaptation and rates of forcefield washout were greater than the rates of forcefield adaptation (Table 6-2). The plateau and maximal error for each condition were also similar in dystonia to controls (values given in Table 6-2, no statistical difference found).

	Visuomotor adaptation					Visuomotor washout				
	Plateau (a)	Maximal error (b)	Learning index (c)	n	mean R^2	Plateau (a)	Maximal error (b)	Learning index (c)	n	mean R^2
Controls	4.02	28.0	0.139	18	0.80	1.49	23.8	0.164	18	0.81
CD	5.15	25.2	0.131	17	0.77	1.56	23.4	0.168	18	0.80

	Forcefield adaptation					Forcefield washout				
	Plateau (a)	Maximal error (b)	Learning index (c)	n	mean R^2	Plateau (a)	Maximal error (b)	Learning index (c)	n	mean R^2
Controls	3.02	22.7	0.222	17	0.61	0.849	20.2	0.369	15	0.57
CD	3.07	20.4	0.200	17	0.62	0.971	25.5	0.389	12	0.58

Table 6-2 Modeling visuomotor and forcefield adaptation and washout.

The number of inclusions (n) out of a total possible of 19 is detailed in the table and the mean R^2 of the remaining group given.

6.3.3 Kinematic variables

There were no significant differences between groups for reaction time (Table 6-3) rmANOVA revealed no significant difference for GROUP $F(1,18)$ = .150, p=.703 or GROUPxCONDITION $F(4,72)$=.633, p=0.604. There was a significant effect of CONDITION $F(4,72)$=14.098, p<.001 however Tukey post-hoc tests following a one-way ANOVA were not significant.

There were no significant differences between groups for movement duration. RmANOVA did not show an effect of GROUP $F(1,18)$=0.641, p=0.434 or GROUPxCONDITION $F(4,72)$=.379, p=0.823. A significant effect of CONDITION was observed $F(4,72)$=6.879,

p<.001. One-way ANOVA with Tukey post hoc analysis showed that this effect was due to a significant difference between baseline (highest movement duration) and forcefield adaptation (p=0.006) and forcefield washout (p=0.022).

	Reaction time (ms)		Movement duration (ms)	
	Control	Cervical dystonia	Control	Cervical dystonia
Baseline	459 (23)	405 (21)	305 (11)	315 (11)
Visuomotor adaptation	446 (22)	407 (16)	290 (7.7)	289 (9.2)
Visuomotor washout	414 (20)	413 (18)	288 (6.4)	291 (8.3)
Forcefield adaptation	420 (22)	424 (23)	274 (9.4)	286 (8.0)
Forcefield washout	387 (17)	439 (24)	278 (7.1)	289 (6.8)

Table 6-3 Reaction time and movement duration

Mean values with standard error of the mean shown in brackets.

6.3.4 Clinical correlations

Firstly the severity of cervical dystonia was assessed for any relationship to rates of adaptation and washout. Both the severity subscore of the TWSTRS (visuomotor adaptation r=-.22, p=.401; visuomotor washout r=-.02, p=.938; forcefield adaptation r=-.04, p=.887; forcefield washout r=-.07, p=.832) and the total score of the TWSTRS (visuomotor adaptation r=.13, p=.612; visuomotor washout r=0.7, p=.770; forcefield adaptation r=.002, p=.993; forcefield washout r=.14, p=.658) were correlated and no relationships were found.

Eleven of the 19 patients had clinically apparent head tremor. The dominant frequency of the tremor had a mean of 5.02Hz (SEM 0.331Hz, range 3.1Hz to 7.5Hz) in keeping with previous observations. As there was no clear grouping of tremor severity based objectively on total power of tremor alone we divided subjects into those with clinically apparent head tremor (11 patients) and those without apparent head tremor (8 patients). The learning indices for these two groups were comparable in all four tasks with no significant difference seen (Table 6-4). In the patients with tremor, there was no correlation between tremor severity (log of total power) and the learning indices of each of the four conditions: visuomotor adaptation (r=-.14, p=0.764), visuomotor washout (r=.24, p=0.562), forcefield adaptation (r=.25, p=0.557), forcefield washout (r=-.47, p=0.347).

Condition	Group	*n*	Learning index Mean (SE)	t-test
Visuomotor adaptation	No tremor	8	0.108 (0.019)	$t(15)=1.58$, $p=.134$
	Tremor	9	0.152 (0.021)	
Visuomotor washout	No tremor	8	0.182 (0.018)	$t(16)=-1.18$, $p=.280$
	Tremor	10	0.158 (0.013)	
Forcefield adaptation	No tremor	7	0.189 (0.049)	$t(15)=.188$, $p=.853$
	Tremor	10	0.201 (0.042)	
Forcefield washout	No tremor	5	0.412 (0.048)	$t(10)=-.255$, $p=.804$
	Tremor	7	0.373 (0.125)	

Table 6-4 Learning indices for each of the four conditions.

Mean values with standard error of the mean shown in brackets. Learning indices were only included in comparison if the model suitably fitted the data ($R^2>0.4$). Eleven of the 19 patients had clinically apparent tremor.

6.4 Discussion

In this study we have demonstrated that motor adaptation in cervical dystonia is identical to healthy controls in two tasks which test visual and proprioceptive sensorimotor integration. These data support preserved cerebellar function within this domain.

Motor adaptation is a task commonly used, across species, to directly examine cerebellar function[24,167]. An environmental perturbation introduces a movement error requiring subjects to adapt their performance of a task. The sensory prediction error (how the actual sensory movement outcome differed from the predicted sensory movement outcome) is used to update subsequent motor performance, with this type of learning being strongly dependent on the cerebellum[25]. Interestingly, the cerebellum has not only been linked to the formation of forward models which predict the sensory outcomes of motor commands; it may be that the cerebellum has a role in forming cognitive predictions for non-motor cerebellar functions such as language [168,169]. This argument is supported by the highly conserved structure of the cerebellar microanatomical architecture, which is thought to imply that the computational qualities of cerebellar cortex remain constant[169,170].

Patients with myoclonus dystonia (caused by mutations of the SGCE gene, DYT11) have been shown to have impaired saccadic adaptation[171]. However it is not known whether findings in combined dystonias can be applied to isolated dystonia. In isolated dystonia motor learning/adaptation has been examined in focal hand dystonia using a joystick task[117]. Each trial had a different visuomotor perturbation and a different position of the target and subjects were asked to correct their movement during each trial. No impairment in motor learning was demonstrated but there was impaired retention. However, contrary to the author's conclusions, this suggests a change in the ability of the motor cortex to retain the new memory rather than a cerebellar deficit[24,117]. Our data in cervical dystonia builds on previous work that we performed with a more simplistic visuomotor adaptation task[89]. This current study differs in that we used a purpose built robot which required larger more complex movements recruiting proximal arm and shoulder muscles. We also used a shooting paradigm which does not allow for online correction and modelled data in a manner which we believe optimally assesses for differences in adaptation. The forcefield condition is more relevant to dystonia in which subtle proprioceptive deficits have been described[172]. Furthermore, visuomotor and forcefield adaptation examine distinct (and common) regions of cerebellar function. Within the anterior lobe of the cerebellum, which contains one of the two body representations within the cerebellum, lobules IV and V are thought to be more important for the forcefield task and lobule VI is more important for visuomotor adaptation[161]. Regions in the posterolateral cerebellum (crus I and II) are thought to be required for both tasks[4]. This analysis of the two perturbations with a large number of patients leads us to confidently conclude that motor adaptation is normal in cervical dystonia.

How do our results link in with the growing body of evidence which implicates the cerebellum in the pathophysiology of dystonia? Certainly for cervical dystonia, if there is cerebellar dysfunction, the nature and extent of cerebellar dysfunction remain to be established.

The normal performance in these adaptation tasks that required use of both visual and proprioceptive input was of interest. Although visual processing is normal in cervical dystonia previous studies have described deficits in proprioceptive tasks. Dystonic subjects are less sensitive at detecting passive movements of the fingers[173] and arms are abnormal in their perception of the vibration induced illusion of movement (which is induced by stimulating muscle spindles with a vibration stimulus)[172,174,175]. How can performance in our tasks be normal in the face of such obvious deficits? One possibility is that tests of proprioceptive sensation are mostly static tasks whereas ours were dynamic, involving sensation during active movement. Furthermore the psychophysical tasks described above require sensory processing and decision making at many levels of the nervous system and some of these are likely to be distinct to networks involved in implicit motor tasks. For example higher

order/consciously regulated elements of decision making could have a greater influence on psychophysical tasks.

The question of whether movement in the asymptomatic arm of patients with cervical dystonia is entirely normal perhaps remains to be definitively answered with future experimental work. Some have described abnormalities in kinematic variables recorded during reaching studies similar to the task used in this article (movement time was not matched between groups and thus some of this data is difficult to interpret[173]) and electrophysiologically, abnormalities in inhibition have been demonstrated at many levels of the nervous system concerned with the control of the arm musculature (e.g. abnormal reciprocal inhibition of forearm muscles in cervical dystonia[176]). However, other studies in including ours suggest near normal motor performance[89]. Conservation of motor skill in the arms is the norm with most patients with cervical dystonia and we argue that this is perhaps against a global movement deficit in the focal dystonias.

Our conclusions for dystonic tremor are more tentative. We did not find evidence to support a relationship between the ability to adapt and the severity of dystonic tremor. Secondly, splitting subjects into whether or not they had tremor did not reveal a group difference in rates of adaptation. The pathophysiology of dystonic tremor is poorly understood but many 'primary' tremor models are thought to involve the cerebellothalamocortical network. Certainly in patients with essential tremor, there seems to be multimodal evidence for pathological involvement of the cerebellum (structural imaging[177], functional imaging[178], eye movement analysis[179], deficits in eye blink conditioning[124] and motor adaptation[164].) Here we have performed one of the first studies to examine the role of the cerebellum in the generation of dystonic head tremor and have not yet found a clear interaction. Our findings support studies that suggest different mechanisms between essential and dystonic tremor. For example in essential tremor the second agonist burst during ballistic movements is delayed and this finding is often ascribed to a lack of cerebellar prediction[180]. This delay in timing is not observed in patients with dystonic tremor[181].

A final implication of our results is that the preservation of adaptation, a type of motor learning, may have potential therapeutic implications. Adaptation could be used to reduce errors in dystonic movements and this could translate into advances in physical therapy for dystonia[182].

A limitation of our study is the possibility that our task was insensitive to a deficit in adaptation. Perhaps errors were too large in our task to detect cerebellar dysfunction within a biologically relevant range. Against this is the observation that patients with cerebellar damage had an equal difficulty with small and large perturbation errors[183]. Furthermore, based on our mean and variance from the visuomotor adaptation condition (effect size

0.097) and assuming a power level of 0.8 we would need approximately 2700 subjects in total in order to achieve a significant result. Therefore we do not believe our null results are due to a lack of power. Another perhaps unavoidable limitation is that patients were receiving botulinum toxin injections (the mainstay of treatment for cervical dystonia). We tested patients when maximally symptomatic prior to injections but the long-term influence of botulinum injections on results cannot not be fully assessed in this or other studies that have used an identical approach.

7 Adaptation in DYT1 dystonia

7.1 Introduction

Dystonia is a movement disorder characterised by abnormal posture which is often diagnosed based on clinical observation alone. Interestingly, the diagnostic algorithm which the clinician implements to reach this conclusion and decide on dystonia rather than another movement disorder is poorly defined. Which specific aspects of posture and movement are affected remains poorly understood[184]. Most of the characterisation of *how* movement is abnormal in dystonia is based on a series of studies examining children with heterogeneous aetiologies (idiopathic, neurodegenerative, secondary to injury at birth). These children reproducibly show that increased movement variability is a core feature[185-188]. Increases in movement variability in dystonia are thus thought to reflect the dystonic disease process, assumed to be detrimental to motor control, and at some level translate into the movement disorders we observe in clinical practice.

However more recently it has been appreciated that motor variability is not just noise and, at least in part, represents a useful information source for the motor system. Wonderful studies in songbirds show that young birds inject 'noise' or variability into their song when the requirement is to optimise learning conditions but immediately dampen such noise when high accuracy of song is required to perform to a potential mate[189,190]. Moreover, a similar dynamic regulation of variability can also be shown in humans performing motor tasks under different experimental manipulations and greater variability of baseline movement parameters, relevant to the subsequent learning task, strongly predict better motor learning[191].

How do we juxtapose increased variability being a predictor of both a clinical movement disorder and an optimiser of motor learning? We propose that the positive relationship between variability and learning observed in health must saturate at some point. Presumably if the normal dampeners on variability are dysfunctional, as is suggested by previous data in children with dystonia, there is a threshold at which high variability starts to impair learning.

We explored this hypothesis in DYT1 dystonia. We first wished to confirm that DYT1 dystonia, like mixed cohorts of childhood dystonia, was a disorder characterised by increased movement variability. This monogenic form of dystonia is caused by a single mutation in TOR1A, and is characterised by an isolated dystonia with no additional symptoms or signs other than tremor[192]. As such this is an ideal group within which to try and define quintessential features of dystonia motor control. If in DYT1 dystonia

physiological boundaries of variability which exist in health are exceeded one would predict such variability to be to the detriment of motor learning; a reversal of the patterns seen in health.

7.2 Methods

7.2.1 Subjects

Ten patients with generalised DYT1 dystonia (mutations confirmed) and 12 age-matched controls were recruited (Table 7-1). All were symptomatic in the right arm and most were taking medications. Those receiving botulinum toxin injections were tested at the end of their therapeutic window (minimum 3 months post last injection) and none had received deep brain stimulation. Subjects had no additional neurological/musculoskeletal problems of the arm or significant cognitive impairment.

Age (yrs)	Sex	Hand	Tremor	Severity right arm (0-16)	Severity total (max 120)	Duration (yrs)	Medication
59	M	R	Yes	6	38	50	diazepam, baclofen, botulinum toxin (> 3 months, paraspinal)
69	F	R (L)	Yes	9	46	58	trihexyphenidyl, clonazepam
24	F	R	No	6	16	15	botulinum toxin (> 3 months forearms)
24	F	R	Yes	6	32	2	trihexyphenidyl, clonazepam
42	M	R	Yes	1	8	16	trihexyphenidyl
44	F	R	No	2	29	34	trihexyphenidyl, clonazepam
48	F	R (L)	No	6	14	40	trihexyphenidyl, clonazepam
46	M	R	No	2	15	13	botulinum toxin (>3 months, paraspinal)
50	M	R (L)	No	12	55	42	trihexyphenidyl, botulinum toxin (> 1 year),
33	F	R	No	6	16	22	botulinum toxin (bilateral lower limb, > 1 year)

Table 7-1 Patient characteristics

Hand preference is documented at the time of the study (if different, hand preference during childhood is given in brackets). The duration of symptoms (from onset to current age) is given in years. The Fahn-Marsden Motor Score for the right arm (maximum severity = 16) and total score (maximum severity = 120) were calculated. Time since last botulinum toxin injections are given in brackets and was always greater than 3 months. There was no significant difference between patients and controls in respect to age (mean patient age 43.9 (± 14.3), mean control age 42.3 (±13.8), t(18) = -0.223 p=0.826). Patients were recruited from the National Hospital for Neurology and Neurosurgery.

7.2.2 Task

Participants were seated with their forehead supported on a headrest. Their semi-pronated right hand gripped a robotic handle underneath a horizontally suspended mirror. The mirror prevented direct vision of the hand and arm and showed the reflection of a computer monitor mounted above. The central starting position was marked by a square (1.5cm) and the position of the manipulandum was indicated by a circular cursor (radius 0.3cm). For each trial to be initiated the cursor had to be within the central starting position. A square target

(1cm) subsequently appeared randomly in one of four radial locations 6cm from the centre point (directions: 45°, 135°, 225°, 315°). Subjects were instructed to make a fast movement towards the square and to stop at the target. Movement time was set at 1s. Previous studies suggest that both *feed forward* (at maximal velocity, prior to availability of sensory information) and online *feedback* learning mechanisms can be assessed by such pointing movements[27]. At the end of each trial the robot (passive movement, patient asked to relax) returned the hand/manipulandum to the centre and visual feedback of cursor position was removed until back within the central square (with the aim of minimising additional feedback during this phase).

Participants familiarised themselves with the robot and basic task by performing 25 trials during which verbal feedback was given to further explain the desired movement (data not analysed). Each subject then completed 3 different experimental conditions: a baseline block consisting of 96 trials, an adaptation block of 192 (2x96) trials and a washout block of 192 trials (Figure 7-1). Adaptation was measured in response to a visuomotor perturbation in which visual feedback was distorted by 30° in the clockwise (positive) or anticlockwise (negative) direction. The direction of the perturbation was randomised across subjects. The total time of the experiment was approximately 25 minutes.

7.2.3 Analysis

Analysis was run using custom written matlab scripts (Matlab R2015a, TheMathWorks). To avoid sensitivity to outliers, any trials in which the total radial distance travelled was <2cm (incomplete movement), or the angular error was greater than 60° in either clockwise or anticlockwise direction at maximal velocity or the end of the trial (likely error in identifying target reach direction) were excluded. This excluded 1.47% of trials in controls, and 1.58% of trials in subjects with DYT1 dystonia.

Movement characteristics during the baseline block were quantified using the following methods. *Pathlength* was the total distance travelled from the start to the end of the trial a parameter which is elevated with any non-efficient deviation of trajectory from start to finish. To investigate the spatial distribution of error during the baseline block 95% confidence ellipse of the scatter of cursor position were calculated at (i) maximal velocity (feed forward only) and (ii) the end of trial (with feedback). The ellipses are obtained by applying principal component analysis to determine the direction of maximum and minimum dispersion of distribution in the x-y plane. The eigenvectors of the covariance matrix are the axes of the ellipse, while the lengths of the axes are the corresponding eigenvalues. We calculated three parameters to fully describe each ellipse. The *aspect ratio* was the square root of the ratio of the two eigenvalues (the larger divided by the smaller) as a measure of the shape of the ellipse. The *orientation deviation* was the orientation of the largest eigenvalue relative to the

target direction. Since this measure has low reliability for distributions that are approximately circular we multiplied the orientation deviation by the (aspect ratio-1) which weights each data value by its reliability[193]. The total variance was estimated by the *area* of the ellipse (pi multiplied by the axes of the ellipse, see Figure 7-3).

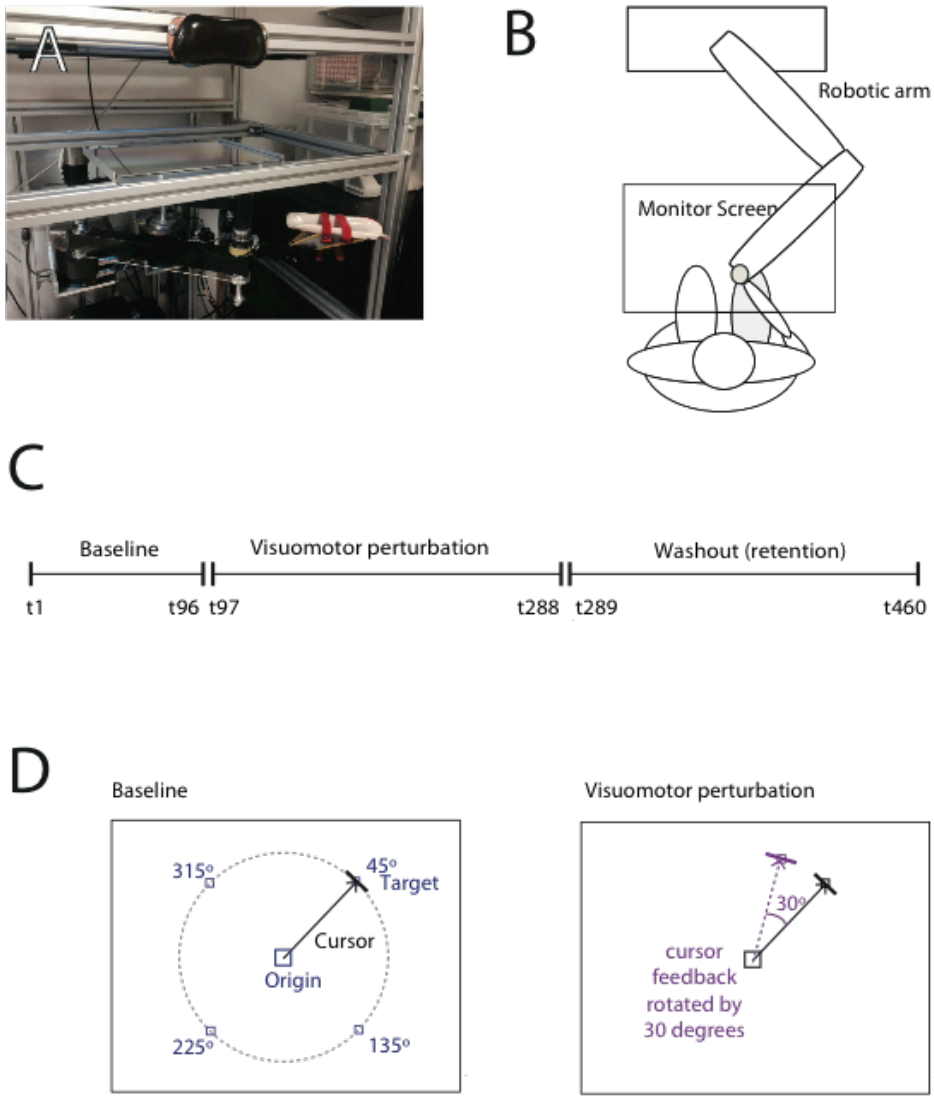


Figure 7-1 Experimental design

A, B *Robot setup.* ***C*** *Structure of experiment: after the baseline block (96 trials), subjects experienced the visuomotor rotation for two blocks (2 x 96 trials), and its subsequent removal over the final 2 blocks (2 x 96 trials).* ***D*** *During baseline movements to four different target directions from the cursor origin at centre were recorded. The order of target location was pseudorandomised such that subjects made an equal number of movements to each quadrant in each block. Subjects were instructed to make a rapid movement to the target. The visuomotor perturbation consisted of the rotation of visual feedback by 30° in either a clockwise or counter-clockwise direction (randomly assigned).*

In addition *reaction time (*time point at which 30% of maximal velocity was first exceeded), *force (*pythagorean of x- and y- forces calculated at all time points, median value for each

trial used) and *maximal velocity* (magnitude) were calculated. Units used throughout are centimetres (cms), degrees (°), milliseconds (ms), velocity in meters per second (m/s) and force in Newtons (N). Movement parameters were calculated for each trial and median and standard deviation across the baseline block were used to compare central tendency and the variability respectively.

Learning and retention were assessed during the application and removal of the visuomotor transformation respectively. Feed forward motor control and adaptation were characterised by examining the angular error at maximal velocity. Online contributions to corrective mechanisms were estimated by subtracting the angular error at maximal velocity from the angle of the cursor at the end of the trial. Each of these parameters were assessed at three different time points. '*Early'* was calculated by taking the mean of the trials 2 to 17 from the onset of the perturbation. '*Late'* was the mean of last 16 trials before visuomotor perturbation removed. Memory for adaptation, *retention*, was assessed during the first 16 trials after the perturbation had been removed. As the total magnitude of adaptation obtained varied significantly between subjects retention was normalised by the late adaptation value for each individual before group comparisons were made.

To examine how baseline variability interacted with learning and retention we firstly examined variability which was relevant to the systematic visual transformation: the standard deviation of angular error at maximal velocity (task-relevant variability). The standard deviation of force applied to the robot handle across trials was considered to be variability without direct relevance to the subsequent learning task. The DYT1 group were classified as either a low or high variability group ($DYT1_{low}$ and $DYT1^{high}$) by a median split of task-relevant variability.

7.2.4 Statistics

IBM SPSS Statistics was used for a statistical analysis. Due to the small sample size we used the non-parametric Wilcoxon Rank Sum test to compare the two groups. Repeated measures ANOVA (rmANOVA) was used to compare confidence ellipses parameters across the four reach directions (repeated factor) with group as a between subject factor. One-way ANOVA with three subject groups (control, $DYT1_{low}$ and $DYT1^{high}$) was used to compare mean values of adaptation, end error and online learning. Bivariate and partial (controlling for severity of DYT1 dystonia) correlations were used to assess for covariance between parameters.

7.3 Results

7.3.1 Baseline movement characteristics

Reaches for four example subjects are shown during the baseline block in Figure 7-2A (example subjects from both groups with the lowest and highest median pathlength). Controls demonstrated a stereotyped reach strategy in the large majority of trials and there was little difference between the subjects with lowest and highest median pathlength. In DYT1 there was more variability within the group and more erratic reach behavior was seen in those with increased pathlength. At the group level (Figure 7-2B) median pathlength was significantly increased in DYT1 dystonia (T=100, p=0.012, r=-0.53) and on a trial by trial basis intra subject variability of pathlength was increased compared to controls (T=106, p=0.035, r=-0.45).

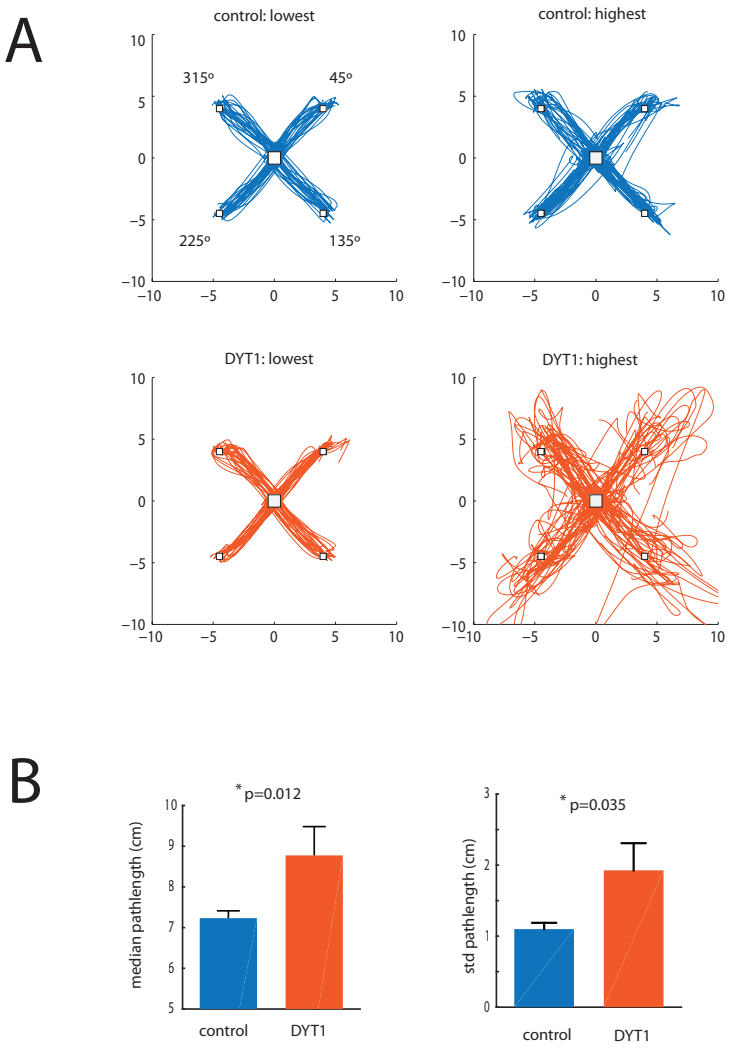


Figure 7-2 Pathlength during baseline block

A *All trajectories for the baseline block in four subjects representing the range of pathlength for controls (blue) and DYT1 (red). The individuals with lowest and highest median pathlength are shown*

in both groups. ***B*** *Median and standard deviation (std) of pathlength were significantly increased in the baseline block.*

7.3.2 Impaired feed forward rather than feedback control

To assess which aspects of motor control were associated with increases in pathlength in DYT1 we looked at the spatial scatter of movements at (i) maximal velocity and (ii) at the end of the trial. Maximal velocity assesses feed forward motor control (movements not under the influence of online sensory feedback) whereas at the end of the trial there has been sufficient time for online feedback to have been integrated into movement corrections. The scatter of position at *maximal velocity* in an example subject is shown in Figure 7-3A. To quantify the characteristics of this distribution for each reach direction we fitted confidence ellipses which enclosed 95% of the data points as detailed in the methods. Interestingly the aspect ratio of the confidence ellipses and the orientation of reach relative to target direction were not significantly changed in DYT1. However the mean area of the ellipse was increased (rmANOVA over four reach directions, effect of group, $F(1,20)=5.75$, $p=0.026$, $r=0.47$, partial $\eta^2=0.202$).

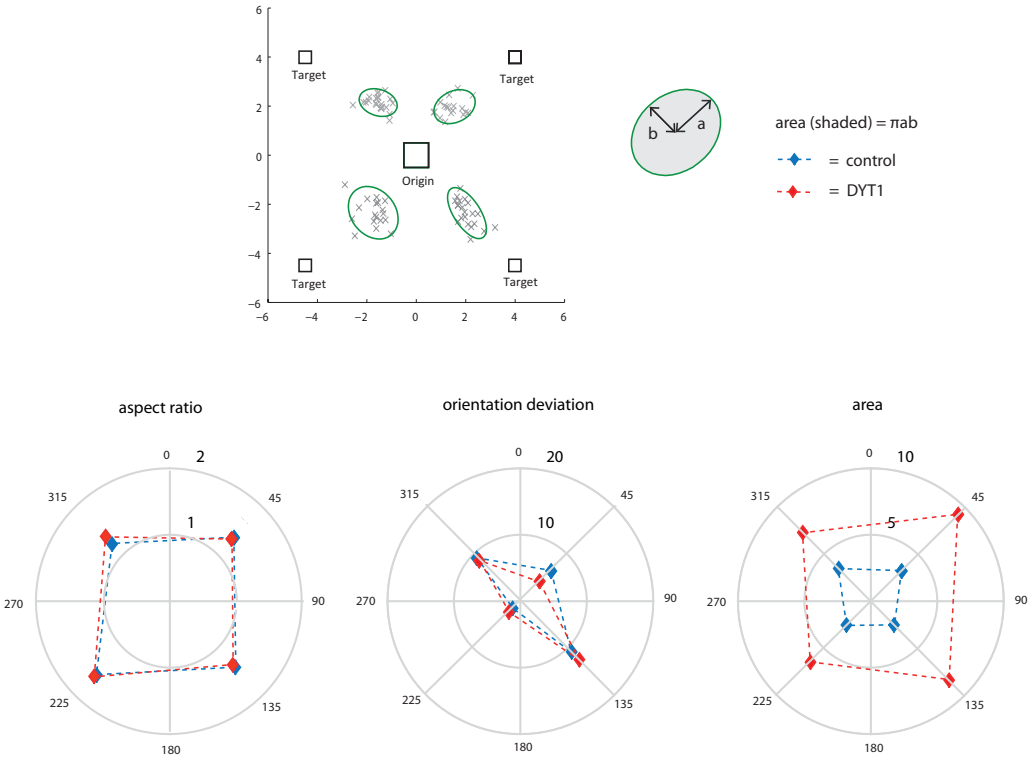


Figure 7-3 Movement features at maximal velocity

A *Scatter of position at maximal velocity during the baseline block from an individual subject is shown (each cross an individual trial). Confidence ellipses encompassing 95% of the variability were calculated for each reach direction and the aspect ratio, angle of ellipse relative to reach direction and the area of the ellipse were derived.* ***B*** *Polar plots for ellipse parameters are shown plotting each value*

relative to its reach direction (45°, 135°, 225° and 315°). The area of the ellipse, or scatter/variability of the x-y position was significantly increased in dystonia.

These effects were selective as the mean area of ellipses at the *end* of trials was not different between groups (rmANOVA over four reach directions, effect of group ($F(1,20)=2.133$, $p=0.15$, partial $\eta^2=0.16$). Nor were there any other changes in the ellipse distribution at *end* of trial as defined by the aspect ratio and reach direction. In addition, other parameters in the baseline block such as reaction time, magnitude of maximal velocity and force exerted were no different between groups (see Table 7-2).

age (yrs)	control	DYT1	Wilcoxon signed rank test
reaction time (ms)	404 (15.3)	423 (16.4)	*T*=130, *p*=0.62, r-0.10
force (N)	5.53 (0.94)	5.24 (1.38)	*T*=99.0, *p*=0.29, r=-0.22
maximal velocity (m/s)	0.89 (0.0036)	0.98 (0.073)	*T*=114, *p*=0.11, r=-0.34

Table 7-2 Median (SEM) reaction time, force and maximal velocity during baseline block.

7.3.3 Impaired adaptation learning with high movement variability

We then examined whether such increases of variability interacted with learning in response to the visuomotor perturbation. Firstly we looked at variability during the baseline block which could be classified as relevant to the subsequent learning task: the standard deviation of angular error at maximal velocity (task-relevant variability). In healthy controls, no significant correlation was observed between task-relevant variability and the early initial adaption phase (Figure 7-4A: $r^2=0.101$, $p=0.312$) or the later phase, a measure of the total adaptation achieved (Figure 7-4B: $r^2=0.013$, $p=0.716$). However in DYT1 dystonia as task-relevant variability increased the ability of the individual to adapt decreased. Baseline angular variability negatively correlated with both the early initial rate of adaptation (Figure 7-4C: $r^{2=}0.803$, $p<0.001$) and the magnitude of late adaptation achieved (Figure 7-4D: $r^2=0.648$, $p=0.005$). This effect was influenced but not fully explained by clinical severity (partial correlation with severity as cofactor for late adaptation, $r^2=0.808$, $p=0.012$). Such relationships between variability and learning were not seen for other qualifiers of movement variability. For example if the variability of force applied to the robotic handle is examined, a parameter less relevant to learning the adaptation task, baseline force variability shows no relationship to subsequent rates of learning.

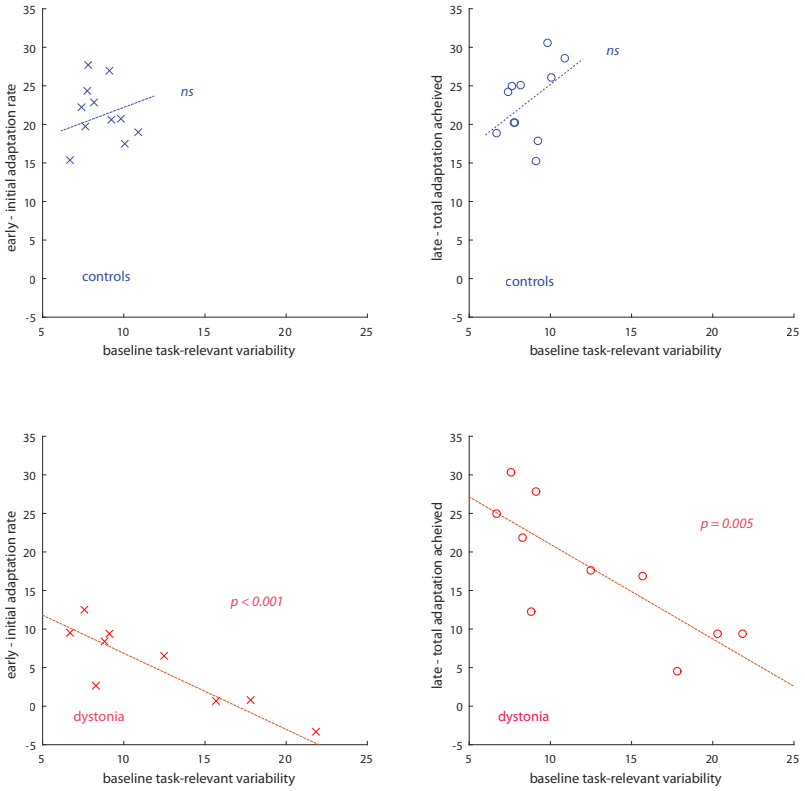


Figure 7-4 Baseline task-relevant variability and adaptation in DYT1 dystonia

The standard deviation of angular deviation from target direction at maximal velocity was used as an indicator of motor variability with task relevance. A, B In healthy controls, such variability, was not detrimental to learning. C,D In DYT1 dystonia; as the magnitude of variability increased the ability of the individual to adapt decreased both for the early initial rate of adaptation and the later phase which estimated the total adaptation achieved.

7.3.4 Deficits in adaptation partially compensated by greater online corrections

A median split of the DYT1 group by task-relevant variability into either low or high variability group ($DYT1_{low}$ and $DYT1^{high}$) exemplifies the implications of this finding further. In Figure 7-5A adaptation learning is plotted in epochs of 8 trials. In $DYT1^{high}$ the initial rate of adaptation was reduced in comparison to both the control group and to $DYT1_{low}$ (ANCOVA with severity of dystonia as a covariate, between group effect: $F(3,18)=6.8$, $p=0.003$, partial $\eta^2 = 0.53$, post hoc group wise comparisons are shown on the bar charts). Furthermore the total magnitude of adaptation achieved at the end of the perturbation block was reduced in DYT1 dystonia (ANCOVA $F(3,18)=4.5$, $p=0.016$, partial $\eta^2=0.43$).

The direction of the cursor at the end of the trial (end angle, Figure 7-5B did not show a significant difference between groups at the start of the perturbation ($F(3,18)=0.30$, $p=0.83$, partial η^2 0.047). However a difference was observable at the end of the perturbation ($F(3,18)=8.9$, $p=0.001$, partial $\eta^2=0.60$, post hoc group wide comparisons shown on bar charts). An estimation of the online learning contributions is shown in Figure 7-5C. Online

feedback mechanisms are used to a greater extent by all groups during the start of the perturbation adaptation mechanisms gradually recalibrate the motor system in response to the visuomotor perturbation. In addition it can be seen that in DYT1^{high} increased recruitment of online learning mechanisms partially offset deficits in adaptation learning during this early phase (ANCOVA F(3,18)= 3.8, p=0.027, partial η^2=0.39). At the end of the perturbation this effect was less and not statistically significant (ANCOVA F(3,18)=1.4, p=0.27, partial η^2=0.19). Retention of adaptation was no different between groups (ANCOVA F(3,18)=1.40, p=0.27, partial η^2=0.19) once the perturbation had been removed.

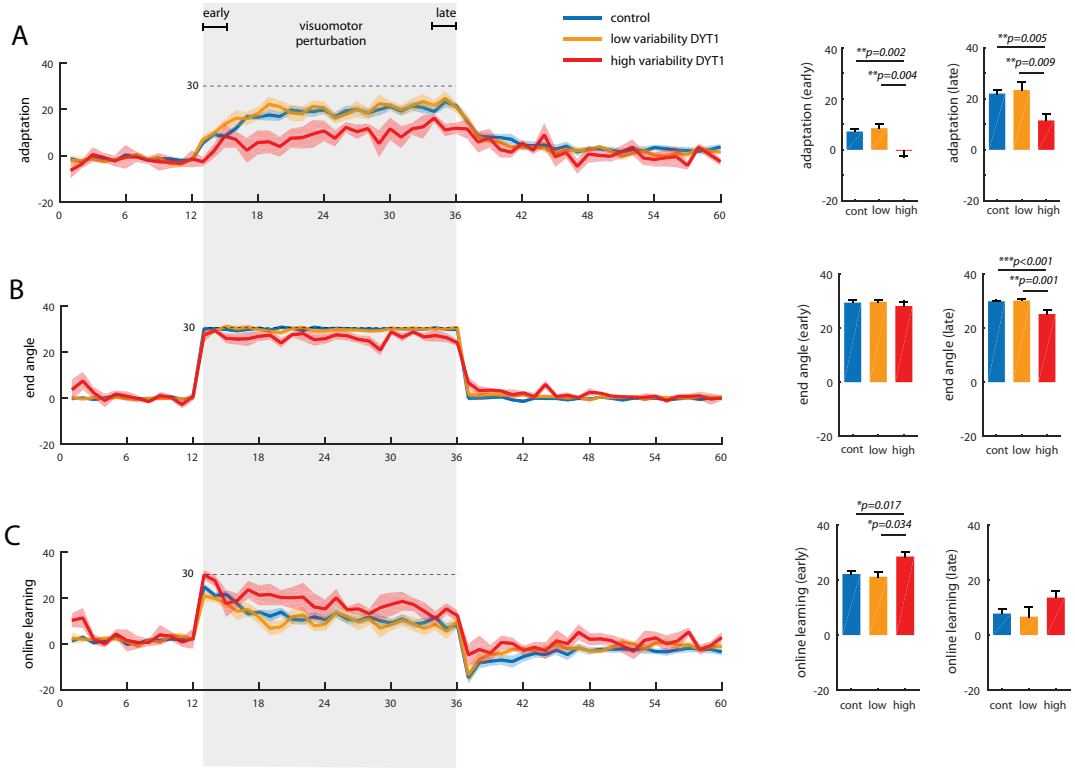


Figure 7-5 Different ratios of learning in DYT1 with low versus high levels of variability

A *Learning parameters are plotted in epochs of 8 trials. When the perturbation is applied all groups gradually adapt steadily until a plateau of this learning is reached. In DYT1^{high} the initial rate of adaption ('early') and the total magnitude of adaption ('late') achieved was reduced.* ***B*** *End angle in DYT1^{high} was less accurate at the end of adaptation, significantly underachieving the optimal angle of 30◦ (see bar chart to the right)* ***C*** *Estimate of online learning contribution to task performance. The deficit in adaptation learning in DYT1^{high} is partially compensated by an increase in the magnitude of online learning. Bar plots to the right of the lineplots demonstrate differences in the ratio of learning mechanisms recruited between the three clinical groups during early and late phases of the perturbation blocks.*

7.4 Discussion

One of the first studies of dystonic movement, published in 1989, commented that the basic motor programs are relatively preserved in this condition[194]. Unlike other neurological disorders such as Huntington's and Parkinson's disease kinematic abnormalities in dystonia, although present, were more difficult to define[195]. A series of detailed studies in children with generalised dystonia of mixed aetiologies have shown dystonic movements to be slower (and with altered speed-accuracy trade-offs) and more variable[185-187,196]. We were interested in whether the pure dystonic condition DYT1 dystonia (with no spasticity or other complicating neurological deficits), would show similar movement abnormalities. We found selective features of movement to be more variable in DYT1 dystonia. Pathlength, which is elevated with any non-efficient deviation from start to finish, was increased DYT1 dystonia and its variation within dystonic subjects on a trial-by-trial basis was increased. Furthermore at maximal velocity, a marker of feed-forward control, the spatial distribution of movements was greater, an effect which was not observed by the end of the trial. Our task had a fixed movement time and within this constraint we found no abnormalities in timing, velocity or force applied.

In healthy controls it has been shown those with greater baseline task-relevant variability perform better during a subsequent motor learning paradigms[191]. Following the observation that variability was increased in DYT1 dystonia we sought evidence to support our hypothesis once a certain threshold is reached, even task-relevant variability will no longer bestow useful information for the consequent learning paradigm. If its magnitude is too large it may dilute environmental teaching signals (in our experimental this was a systematic 30◦ visuomotor perturbation). The negative correlation seen between angular variability and metrics of adaptation learning in DYT1 dystonia strongly supported this premise. Furthermore when DYT1 were grouped into a low and high variability group, $DYT1^{high}$ were significantly impaired in the rate and magnitude of adaptation compared to controls subjects and $DYT1_{low}$[191]. Adaptation is a fundamental calibration system used continuously by the motor system to update our movements. The finding that this was significant impaired in a stratified group of DYT1 patients when stratified was therefore a substantive finding.

How do these data help inform our understanding of the pathophysiology of dystonia? A critical factor to this discussion is to try and elucidate what is the driving deficit in DYT1 dystonia: does increased variability cause impairments of adaptation or given the role of the cerebellum in motor adaptation are our findings suggestive of cerebellar dysfunction? The basic neural circuitry required for adaptation is shown in Figure 7-6A. A motor command activates the musculoskeletal system to move and sensory consequences of this movement are fed back to the nervous system. However both the motor and sensory system have inherent delays (up to 70ms) which is too slow for the control of fast movement elements. It

is therefore thought that an additional efference copy of the motor command is issued to a forward model which generates *predicted* sensory consequences[29,160,197]. A neuronal comparator subsequently compares the actual and predicted sensory consequences (allowing for the sensory delay) to generate accurate error signals which can be used to update subsequent movements and internal models. The cerebellum is thought to be responsible for the generation of the forward model and/or the predicted sensory consequences[15].

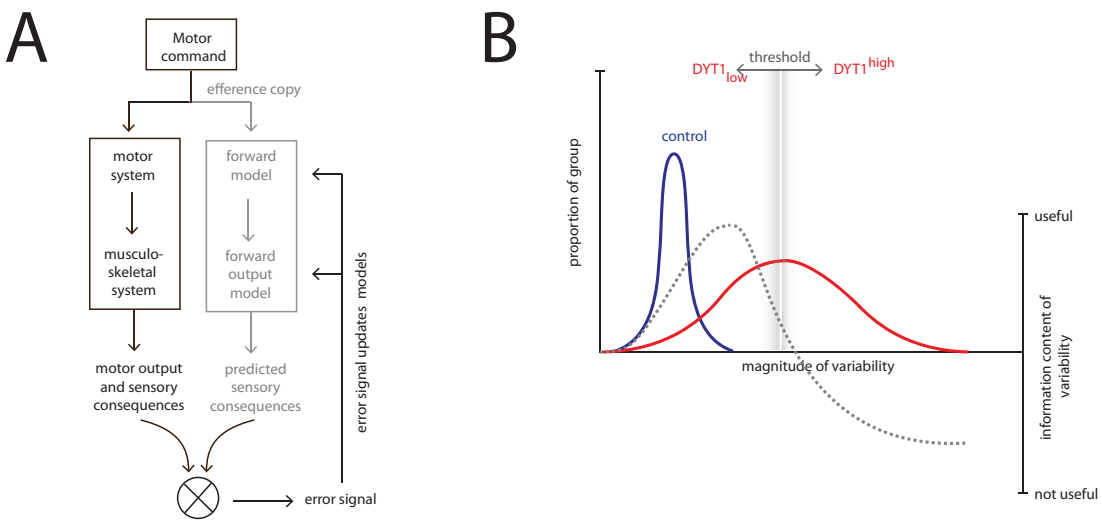


Figure 7-6 Adaptation and the informational content of variability

A Theoretical model for how forward models are implemented. Motor commands directed to the musculoskeletal system are also copied to forward models that mimic these systems. The cerebellum is a core structure involved in forward modeling and predicting sensory consequences. B. In this figure the distribution of variability in controls (blue) and DYT1 dystonia (red) is shown. In controls a positive linear relationship between the magnitude of variability relevant to a subsequent learning task is seen (grey dotted line). This linear relationship is unlikely to be infinitesimal and supposedly in health levels of variability are tightly regulated within this physiological and informative range. In dystonia such control of variability appears to be disturbed. Patients have a greater range and median magnitude of variability. At some point the threshold of useful information content for the motor system is exceeded and forms of learning relevant to that variability parameter may be impaired.

If variability is the primary driver of motor dysfunction in DYT1 dystonia (e.g. noise is injected into part of the motor system responding to the motor command) the actual motor and sensory consequences will be more variable. If such variability is stochastic or unpredictable in nature this could introduce uncertainty into the system. Every time the comparator has to compare the actual and predicted motor consequences any teaching signals used to drive recalibration will be diluted. Alternatively if we consider the primary deficit to be cerebellar in DYT1 dystonia (e.g. cerebellar dysfunction could lead to inaccuracies in the forward model,

causing a similar mismatch at the comparator) the system will get erratic teaching signals via an entirely different mechanism. To add yet more complexity to this debate it is thought that in cerebellar disorders there is both increased motor execution variability and (even when accounting for this execution variability) a deficit of adaptation learning[198].

Since only those in the DYT1^{high} group had experiment deficits in adaptation we choose to stress the role of movement variability as the core dystonic feature. Moreover, there are possible mechanisms behind such movement variability. For example, another field of research in which signal to noise rations are frequently debated as a potential mechanism for dystonia pathophysiology, examines oscillatory behavior across different regions of the nervous system. Pathological enhancement of low frequency oscillatory neural activity has been shown between the motor cortex and basal ganglia of patients with dystonia[199]. Such oscillatory activity could inject noise and variability into the motor system at many potential levels ranging from movement preparation through to execution; a possible neural correlate for increased movement variability.

Overall, the selectivity of the observed impairments in DYT1 dystonia should be emphasised. Patients with poor adaptation learning had increased on-line learning corrections that partially masked deficits in adaptation. This demonstrated a remarkable recruitment of less affected control mechanisms and preserved flexibility of the dystonic motor system. There may be a greater and more diverse stream of sensory information during online processes which allows the nervous system to identify and assign less importance to sensory parameters corrupted by uninformative variability. This reasoning may also feed into putative mechanisms behind alleviating manoeuvres or sensory tricks in dystonia (additional haptic feedback through contact of an affected body part with another object or self improves dystonic motor manifestations) and may also explain why some studies have found dystonic movements to be slower (increased time allows greater online processing). The benefits of physiotherapy for dystonia is increasingly recognised and targeted retraining methodologies which exploit such intact features of dystonic motor control could increase efficacy further. There were also important negative findings in this study. One of the most interesting is that there is no change in aspect ratio of confidence ellipses at maximal velocity. This suggests that certain fundamental motor commands are intact in line with work in children with generalised dystonia showing that the muscle synergies or motor modules recruited for a specific task are very similar to control subjects[200].

Our study was limited by the small number of patients available to study due to rarity of patients with DTY1 dystonia in adult life that have not already been treated by deep brain stimulation. In addition we did not find a statistically significant positive correlation in controls between task-relevant variability and learning as in previously work. This was likely due to

insufficient power and the less complicated experimental design which we felt was necessary in this patient group.

In conclusion, we have shown that patients with the prototypical dystonic disorder, DYT1 dystonia, have movements characterised by increased variability in specific domains. Excessive variability was significantly associated with deficits in adaptation learning, offering insight into possible mechanisms by which movement calibration may be impaired in dystonia. The remarkable flexibility of the dystonic motor system was also demonstrated as deficits in adaptation were partially offset by increased recruitment of online corrective mechanisms with potential implications for rehabilitation.

8 General discussion and conclusions

Research over the last decade has refined our understanding of the neuroanatomical substrates of dystonia. In addition to basal ganglia dysfunction a much wider sensorimotor network has been implicated and within this network the cerebellum is heralded as a core node. Much of the literature linking the cerebellum to dystonia consists of cases in which lesions of the cerebellum are linked to abnormal posture or indirect experimental associations. Better defining the functional role of the cerebellum in the pathophysiology of dystonia could provide a scientific rational for future therapeutic advances, adding further weight to an early neurosurgical literature which advocates targeting the cerebellum and its outflow tracts.

In health the cerebellum has a unique functional anatomy and well established cerebellar experimental paradigms. Components of some of these experimental techniques have even been mapped to the level of individual cerebellar neural types. I therefore applied such paradigms to groups of subjects with dystonia to try and address *how* the cerebellum is functionally involved in the pathogenesis of isolated dystonia. Each technique was selected with the belief that direct inferences about cerebellar contributions to disease state could be made. Results are now brought together and placed in the context of the current literature. After discussing these results I address some of the outstanding questions in this field before drawing tentative conclusions and possible directions for future work.

Many of the studies detailed in my thesis yielded negative results in their primary aim to identify specific cerebellar mechanisms and some unexpected results ensued. One such line of work, investigating the role of plasticity in the pathophysiology of writing dystonia is attached as an Appendix and concludes that non-invasive plasticity techniques have such variability of response that abnormal plasticity may not be a good disease model for task-specific dystonia.

8.1 Cerebellar modulation of dystonic neurophysiology

In the first experimental chapter I presented data suggesting that motor surround inhibition (mSI) is not a cerebellar mediated phenomenon as modulating cerebellar activity using both anodal (enhancing) and cathodal (inhibitory) cDC did not change the profile of mSI responses in healthy controls. Although it is still feasible that in a dystonic patient group different cerebellar mechanisms exist we decided not to further pursue this avenue of research due to developments in the understanding of mSI. Firstly attention (in addition to the theory that mSI is a correlate of aptitude for finger individualisation) appears to modulate

the magnitude of mSI observed[201] (explicit attention on the hand increases mSI). This may partially explain why mSI is reduced in professional musicians as they are likely to attend less to an 'easy' hand task such as mSI but also may influence the magnitude of mSI seen in a patient group in which attention is very much focused on the symptomatic hand. Furthermore a recent meta-analysis of mSI in task-specific hand dystonia has concluded that mSI is not reliably different from controls (mSI is also normal in cervical dystonia)[202].

Variability of neurophysiological response was also a limiting factor when examining the ability of cDC to modulate PAS25 responses, a common plasticity paradigm used in neurophysiological research (chapter 3). In this study the design was such that we set out to reduce excessive plasticity in patients. However patients had such variability in both the size and direction of their plasticity responses that our experimental design was undermined. The technique however, seemed to be efficacious as cDC 'stabalised' PAS25 responses; reducing the magnitude of both inhibitory and facilitatory responses. As such in diseases in which such plasticity mechanisms are consistently and detrimentally excessive this technique could be therapeutic.

8.2 Pavlovian conditioning and millisecond timing

Rates of eye-blink conditioning were equal to aged matched controls in DYT1 and DYT6 dystonia. This was surprising within the context of previously published results showing reduced conditioning rates in focal dystonia. One conclusion is that different subtypes of isolated dystonia have neuroanatomical variability. However we also have to consider that experimental paradigms have a large amount of variability and such variability is often related to factors that may not be fully controlled for.

For example rates of conditioning dramatically depend on age and this is illustrated in Figure 8-1. In the youngest age group mean levels of conditioning at the end of the learning paradigm are 90%. In the oldest age group the mean level of conditioning is closer to 20%. If we reexamine the study in focal dystonia, 8 controls, 7 subjects with cervical dystonia and 5 subjects with task specific dystonia (3 writing, 2 musicians) were tested. Patients had lower rates of conditioned responses as assessed by two factorial ANOVA of block (within subject factor) and group (between subject factor) showing an interaction of block x group ($F(5,90)=5.4$, $p = 0.043$). However the effect is subtle and if age is added as a covariate then variability starts to undermine the statistical effect: ($F(5,85)=2.23$, $p=0.06$). The between-subject effect of age ($F(1,17)=4.22$, $p = 0.05$) was not significant but showed a stronger trend than the factor dystonia ($F(1,17)=3.36$, $p=0.08$). Of note, subsequent studies studying eye blink conditioning in cervical dystonia (and also describing impaired conditioning in cervical dystonia) shared the same control group[133].

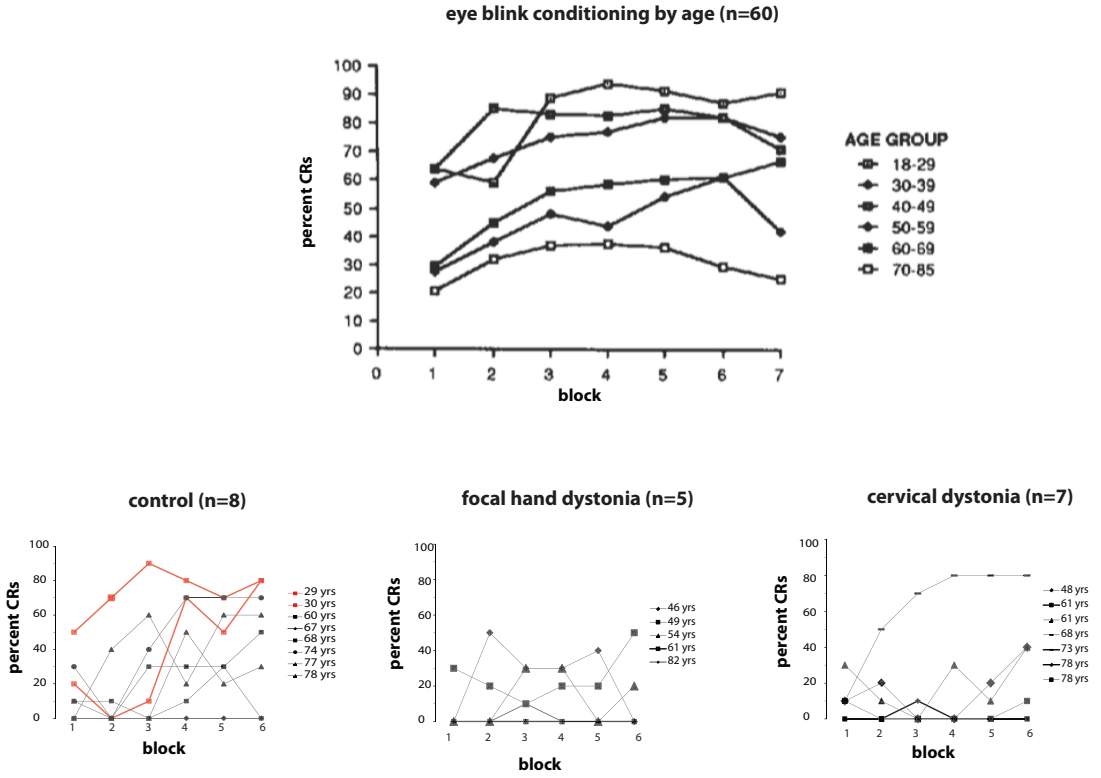


Figure 8-1 Eye blink conditioning - influence of age and focal dystonia

All graphs show the percentage of conditioned responses (CRs) by block on the x-axis. Rates of conditioning dramatically depend on age as shown by this plot of six different age ranges with 10 subjects in each group on the top row of this figure. A plot of the control group (n=8) from the focal dystonia studies shows a large amount of variability within this group and stratification by age is not fully seen due to insufficient power. Outcomes from such studies will dramatically change depending on the subsection of ages contained within the control group. In the control group for example 2 subjects were under 30 years old and this age range was not represented within the dystonic group (top panel adapted from Solomon et al.,[128] and thank you to Dr James Teo for sharing focal dystonia dataset).

Clearly further studies with larger group sizes fully assessing and matching the influence of age would be very informative in this regard. A smaller methodological issue is that currently the scoring of whether or not conditioning has occurred is done by the experimenter and without predefined criteria is highly subjective (and possibly influenced by the prior hypothesis held). In a recent multicenter study we looked at eye blink conditioning and ensured that the rater was both blinded to group and that the score was a mean across 2 independent evaluators - we found no difference in conditioning between DYT11 and controls[203]- and this is a subgroup with impaired saccadic adaptation (see below)[171].

I also explored millisecond timing mechanisms using psychophysical techniques. The temporal discrimination threshold is elevated across subtypes of dystonia using a staircase design. However such paradigms may test a range of sensory (timing, detection of change) and non-sensory (decision making strategy) factors. I tested both temporal resolution and interval discrimination in the millisecond time as abnormalities of either of these could suggest cerebellar mechanisms (Chapter 5). Interestingly millisecond timing mechanisms were intact and results instead pointed to a different decision making strategy in cervical dystonia. The neuropsychiatric profile associated with cervical dystonia sits very much within a network hypothesis for dystonia implicating wider cortical regions. However overall, this study failed to provide mechanistic insight into how the cerebellum contributes to dystonic pathophysiology.

8.3 Adaptation in dystonia

Is adaptation impaired in dystonia? The first publication to study adaptation within the dystonia literature was in a group of patients with myoclonus dystonia with confirmed mutations in the epsilon-sarcoglycan gene (DYT11). Using this oculomotor paradigm they cleanly showed that patients adapted at much lower rates (despite the absence of eye signs in this group on routine eye examination). These results are similar to those seen in essential tremor another disease in which cerebellar dysfunction is potentially implicated[204]. Would such a deficit also be apparent is isolated dystonia?

In chapter 6 we show that in cervical dystonia the cerebellar circuitry involved in two different types of adaptation paradigms (visuomotor and forcefield) appears intact. In addition, the rates of adaptation did not co-vary with presence or severity of tremor. Two other studies have since been confirming preserved adaptation in this group (one with a hand joystick version of visuomotor rotation and another with a split-treadmill task[89,205]).

In chapter 7 I explored adaption in DYT1 dystonia. At a group level there were no differences in adaptation level but if patients with DYT1 with high motor variability were compared to patients with low motor variability adaptation was reduced. For DYT1 dystonia it seems that increased movement variability is the primary finding. Presumably if teaching signals within the adaptation circuit are corrupted with increased noise then this subset of motor control deteriorates (adaptation learning was more sensitive than online learning to such variability). It is only with further research that we can clearly delineate if such variability is generated at a specific site, or if it's the consequence of dysfunction at multiple locations/levels within the dystonic network.

Gait adaptation has also been studied in blepharospasm[205] (impaired) and writing dystonia (impaired in one study, intact in another[205,206]). However again, it may be that additional

factors should be used to aid our interpretation. Firstly, biological feasibility. Writing dystonia is a task-specific dystonia with its pathophysiology intricately linked to the skill which produces the symptoms. It remains unlikely (although not impossible) that patients that are asymptomatic in gait are impaired at gait adaptation, a fundamental continuously active motor calibration. Secondly, the influence of potentially confounding influences may also be underestimated. For example in blepharospasm the eyes intermittently close and this has multiple potential repercussions for motor control and the psychology with which we attempt any task. Such influences are suggested by the lower walking speed (not only adaptation effected) and lower balance confidence (psychology) in the blepharospasm group making the functional significance of the adaptation deficit less certain.

Overall I think the balance of evidence suggests that impaired adaptation is not a core mechanism contributing to isolated dystonia. It has never been implicated in a symptomatic limb or body region and if this learning mechanism is indeed intact then such types of motor learning may be useful in adjuvant rehabilitative approaches to existing pharmacological and surgical therapies.

8.4 Overview of results

My application of the 'purest' cerebellar paradigms have not provided further information on how the cerebellum functionally contributes to dystonia pathophysiology. I have presented good evidence that fundamental computations such as adaptation and associative learning are intact in various groups of isolated dystonia. Such a conclusion is seemingly at odds with an expanding literature that heralds the cerebellum as a key node within dystonic pathophysiology. In the next section I outline some of the reasons why there may be such a divergent literature and the complexities that will inevitably emerge if we are not specific in our research question.

8.5 What is the role of x in y?

What is the role of the cerebellum in dystonia? This becomes a more complex question if x (the cerebellum) and y (dystonia) are loosely defined entities. Furthermore if the probe or experiment (e) that is used to explore the relationship between x and y is indirect, non specific and/or poorly understood then results from experiments addressing this initially simple question will become increasingly complex to integrate.

8.5.1 What cerebellar role (x)?

The most simple manner to approach this question is to consider the cerebellum as a simple switch (single node, binary qualifier for function, top row of Figure 8-2); if the dystonia switch

in the cerebellum is 'on' dystonia manifests and if the switch is 'off' dystonia is not seen. This viewpoint is likely to be too simplistic as exemplified by the DYT1 mouse model discussed earlier in which the animal did not exhibit dystonia when the TOR1A mutation was expressed in the hindbrain alone[42].

Now we could consider dystonia to be a cerebellar disorder but add in further factors that describe the manner in which it needs to be affected in dystonia (single node, multidimensional qualifiers or tuning functions, Figure 8-2). This perhaps better fits with the simple clinical observation that a typical patient with dystonia does not exhibit classical cerebellar signs. Loss of function caused by lesions in the cerebellum typically manifests as ataxia. It has been suggested that dystonia instead represents a cerebellar state in which dysfunction of the cerebellum is more important and that such dysfunction may be precipitated by a lesion causing 'irritation' within the brain[4].

Another consideration is whether there is focal pathology or uniform pathology. The cerebellum has different functions assigned to different regions and topographic representations of the body within sensorimotor regions. Alternatively there could be regionally specific functions of the cerebellum more sensitive to a diffuse pathophysiology (e.g. high spatial-temporal encoding requirement for the control of movement).

However, the cerebellum is intimately interconnected with the basal ganglia and it can be assumed that dysfunction in either will induce change in the other (two nodes, covariance,). It is not known if they act in synergy or whether one partially compensates for the other. Furthermore this relationship could clearly change with time as the disease process changes or the ability to compensate becomes saturated. If we then add in the multiple other putative nodes involved in the pathophysiology of dystonia then the problem again greatly increases in complexity.

Another interesting and unresolved questions is whether the nodes within the network are considered as part of a network hierarchy or not. Many studies for example studying the neurophysiology of the motor cortex assume that this is the common output for the dystonic process. However in torticollis multiple subcortical pathways directly innervate brainstem nuclei supplying the neck muscles[4]. Some postural neck reflexes presumably operate routinely without direct cortical input. It is perhaps more realistic that each node should be considered with equal importance without predefined ranking or order until the relative status of different nodes are better established.

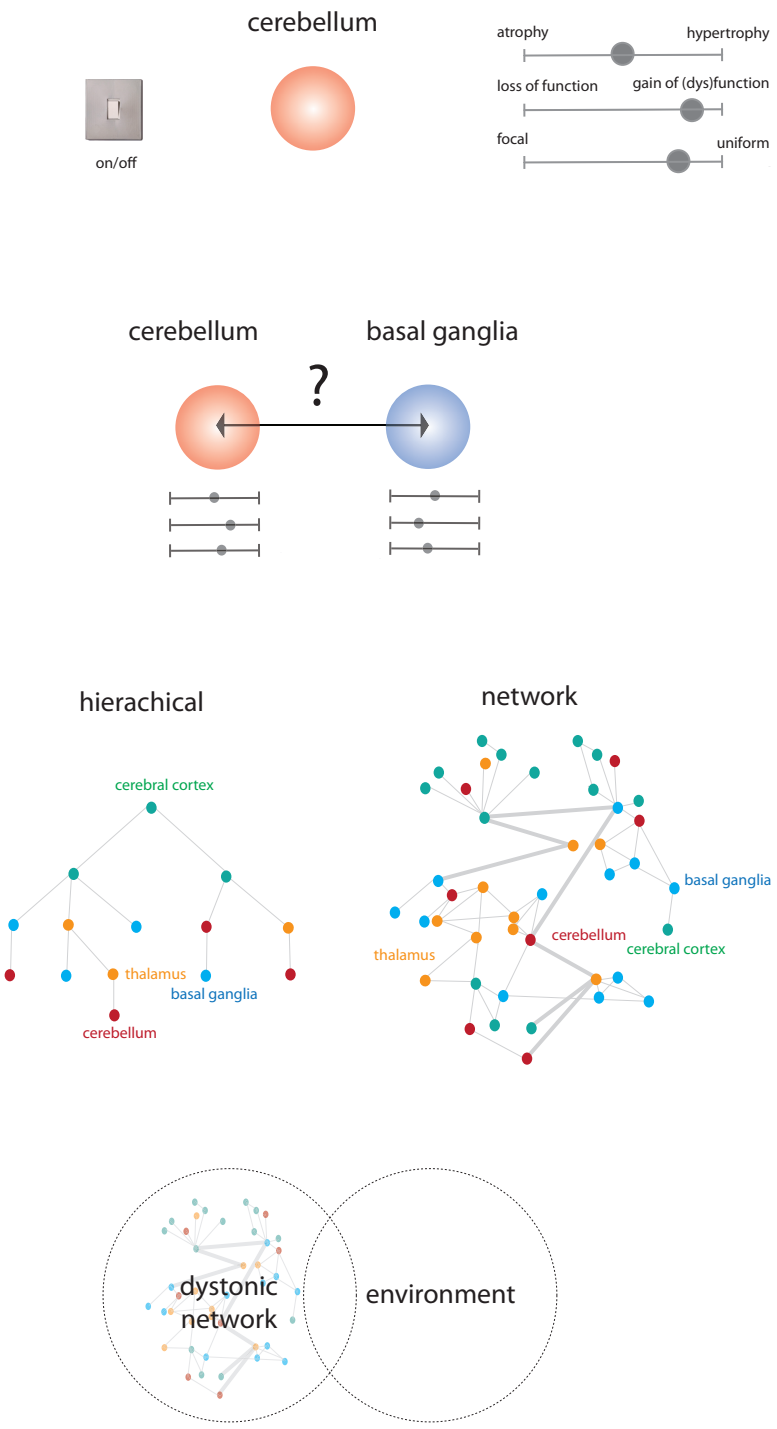


Figure 8-2 What cerebellar role (x)?

Different models for the functional neuroanatomy of dystonia. Most simply dystonia may represent a cerebellar disorder although rather than just an on/off mechanism it may be that certain qualifiers need to be reached before the disease ensues. If a two node cerebellar and basal ganglia hypothesis is discussed a core feature of this model is to determine whether they are both essential and whether their roles co-vary. However the neural regions in which experimental abnormalities have been found are relatively wide. Whether the network model for dystonia has a hierarchical structure is unknown and how environmental factors associated with dystonia interact with this network remains to be fully established.

Finally we should consider the temporal evolution of dystonic phenomenology. Even in the monogenic forms of childhood onset isolated dystonia, postural control and movement is initially intact. It is after major motor milestones are achieved that the dystonic process starts to occur. What mediates this change? There is no overt structural or neurochemical process that has been noted in the brain which we can attribute this time delay to i.e. a concentration of protein that needs to accrue with time before motor impairments are visible. Epigenetic and environmental features could be required in the interplay of disease precipitation e.g. the two hit hypothesis of blepharospasm in which a 'dystonic' predisposition and environmental factors appear to interact. In a similar vein why does a plateau of symptomatology occur in the majority of patients after a certain time period? Has the ceiling of poor postural control been reached for that individual or has a new homeostatic set point been found such that the dystonic insult is now partially stabalised by compensatory mechanisms?

8.5.2 Which dystonia (y)?

Most broadly dystonia can be considered a syndrome of abnormal posture encompassing all subtypes (and assuming equivalence across animal models and humans). As we have seen there is clear evidence that manipulating the cerebellum in animals or focal cerebellar lesions in humans can cause dystonia if such a broad definition is used. However this type of classification would encompass all aetiological heterogeneity and ignore the fact that different subtypes may have different neuroanatomical substrates.

The current classification for dystonia recommends that dystonia can be described along two axes defining their clinical features and aetiology. This thesis has concentrated on exploring the role of the cerebellum in isolated dystonia (previously known as primary dystonia) in which dystonia is the only motor feature (+/- tremor). We have tested patients with cervical dystonia and task-specific dystonia (focal isolated dystonia with onset in adulthood) and also monogenic forms with a generalised distribution caused by mutations in the DYT1 and DYT6 genes (generalised isolated dystonia). As such I prefer the approach of assuming neuroanatomical heterogeneity between the different subtypes until their uniting features are proven and they can then be considered collectively.

This approach is perhaps further justified if we analytically assess the manner by which we describe clinical movement disorders. There are a limited number of dimensions along which we currently classify its disorders to converge on the major movement disorders such as dystonia, chorea, tremor or bradykinesia. This can be interpreted in two major ways. Firstly it may be that there a limited number of stable states of motor dysfunction such that the motor system can only fail in a few different ways (each has its own unique mechanism and neuroanatomy). Alternatively, it may be that the classifiers (clinicians) of clinical

movement disorders have limited ability to discriminate other motor states and our classification may not always be reliable. For example even when eminent specialists (working in the same centre) are asked to classify patients according to their dominant movement disorder (such as dystonia and chorea) there is significant inter-rater variability[207]. Clearly the number of states of motor dysfunction which translate into specific mechanisms is likely to be finite but perhaps current understanding of certain movement disorders such as dystonia is significantly hampered by certain groupings which are irrelevant for disease mechanism. Models for dystonia pathophysiology should therefore try and accommodate differences and similarities among the many different types of dystonia in order to decipher at which biological levels they converge (Figure 8-3).

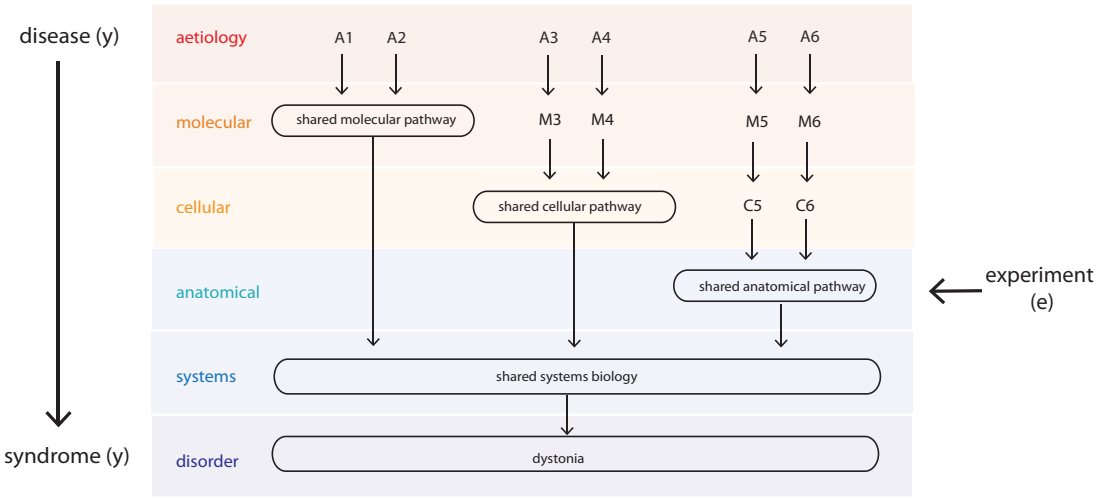


Figure 8-3 Which dystonia (y)?

This conceptual hierarchy accommodates differences and similarities among the different types of dystonia. It shows examples of different levels upon which pathophysiology could converge (adapted from Prudente et al., 2014[208]). If dystonia is investigated as a collective syndrome different aetiologies (A1 to A6) may have a different causal neuroanatomy. The results of experiment (e) investigating cerebellar contributions to the disease state may subsequently be mixed. If specific dystonic aetiologies are investigated (for example DYT1 dystonia) the result of the experiment should be true to the disease.

Finally it is interesting to try and decide whether dystonia represents a developmental regression (a loss of postural/movement control which has already been achieved) or whether it is due overlaying of abnormal movements on top of the healthy repertoire. The first suggests an active disruption to neuronal loci causing site-specific loss of function. The second alternative is perhaps more in keeping with a functional impairment in which movement calibration is gradually impaired. Alternatively there could be release of processes which are usually inhibited. These are all quite different models for disease progression and each alternative brings theoretical implications for how we should treat dystonia.

8.5.3 Which experimental technique (e)?

Frequently the complexity of inferring the anatomical basis of dystonia from common experimental techniques is underestimated. For example if an experimental task being used as a marker of cerebellar function actually has a distributed mechanism (which is often the case) how much does it tell us about the neuroanatomy of 3 different diseases with entirely different anatomical substrates? A very simple simulation reveals that abnormal performance on this test is actually not very informative without additional information (see Figure 8-4).

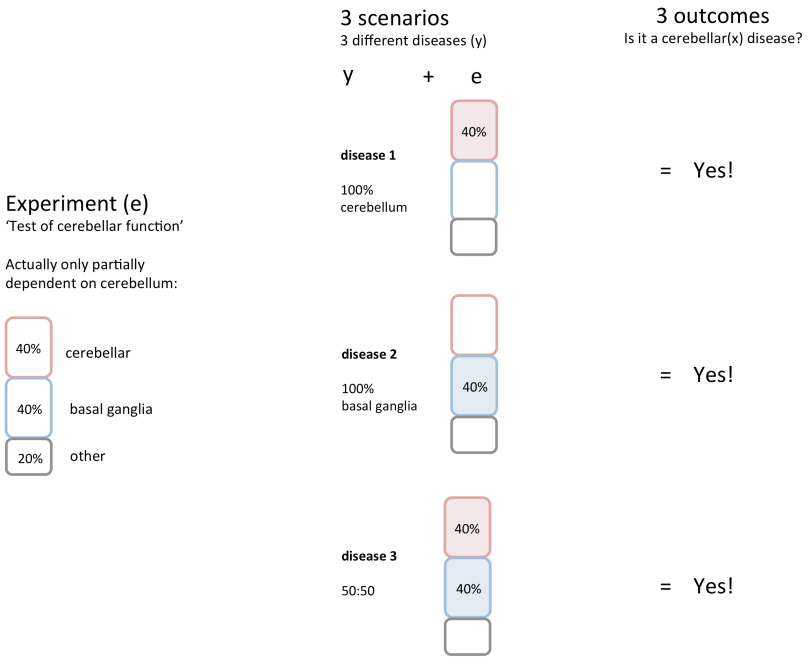


Figure 8-4 Which experimental technique (e)?

In this simulation an experiment (e) is being used as a test of cerebellar function. However actually its neural substrate has a distributed network which can be best estimated as 40% cerebellar, 40% basal ganglia and 20% other regions. This task is applied to 3 different diseases. The first disease is 100% cerebellar and the test is abnormal and the correct outcome is assigned that it is a cerebellar disease. In the 2nd scenario, despite the fact the disease is 100% of basal ganglia origin, the test is still abnormal due to the tests non-specific nature. The wrong conclusion is reached. In the 3rd scenario both the basal ganglia and cerebellum are equally involved (50:50) and again the test is abnormal. This simulation shows that unless an experimental test is specific to cerebellar function, in 3 very different diseases we find out very little about neuroanatomy.

How well we understand our outcome measures is also very important. For example with activation patterns generated by functional MRI (fMRI) increases in activity are often taken to imply dysfunction of a brain region. However such a simple interpretation may be erroneous. For example, neuronal presynaptic activity (thought to be a correlate of fMRI activity) *decreases* in primary motor cortex after skill learning which is thought to indicate increased

efficiency of the neural representation of the behaviour[209]. Would a highly reproduced motor behavior such as dystonic posture become encoded in a similar manner to elicit less population activity? A similar argument of hidden complexity within summary outcome metrics can be made with many experimental techniques.

8.6 Conclusions

"An enormous amount of experimental work has been devoted to the cerebellum but the conclusion drawn from them are so discordant that it seems wise to omit reference to them when possible and to attempt an independent study of the symptoms …. describing them as simply and unequivocally as possible without the use of the conventional terms which have undoubtedly confused the subject"

The cerebellum of man, Gordon Holmes[210]

This is a section of a lecture delivered in 1938 before the Royal Society of Medicine on the role of the cerebellum in man. I wonder whether Gordon Holmes may have similarly reflected on the state of our literature attempting to delineate the role of the cerebellum in dystonia and decided to embark on his own independent study. It remains difficult to draw firm conclusions and integrate all of the evidence which has been published on this topic into a single satisfying framework. What conclusions can be drawn?

I think there is now overwhelming evidence that cerebellar pathology such as stroke, tumor or degeneration can cause a phenotype consistent with dystonia which is similar to the dystonia observed in lesions of the basal ganglia and other regions. As such, an abnormality of posture appears to represents a common final end point for damage or dysfunction within key sensorimotor regions of the brain. However discussing dystonia as a syndrome in this manner is likely to conceal differences between the different subtypes which may also mask important differences of how we should best treat these different diseases. Inferences from this literature cannot be assumed to applied to other dystonic disorders such as DYT1 dystonia or task-specific dystonia which have no gross structural abnormality in any of these brain regions.

I have therefore studied subgroups of isolated dystonia and tried to discuss results within the literature relevant to that particular subgroup (although inevitably subgroups such as cervical dystonia may yet still contain multiple aetiologies within them). What do we know about how the cerebellum contributes to the pathophysiology of *isolated* dystonia?

In terms of basic feasibility, gene mutations responsible for subtypes of isolated dystonia are expressed in the cerebellum. More direct evidence from animals (and the neurosurgical literature) does suggest that the cerebellum has a role in development and/or maintenance of dystonic symptoms. We also have much *associational* evidence (imaging,

neurophysiology) which links the cerebellum to isolated dystonia in humans. We can therefore be confident in the statement that most forms of isolated dystonia appear to be a network disorder and that this network usually includes the cerebellum. However can we be more specific?

I have presented a series of experiments that do not show abnormalities in specific cerebellar functions in a spectrum of groups of isolated dystonia. Such paradigms are thought to be more sensitive than clinical examination to detect cerebellar dysfunction as experimental abnormalities are present in disorders such as schizophrenia and essential tremor despite the paucity of overt clinical cerebellar findings. At present, my results and the results of others do not provide good evidence to implicate specific cerebellar mechanisms as a driver in dystonic pathophysiology despite early work suggesting this may be the case.

8.7 Possible directions for future work

How can we move towards better defining the neuroanatomy of dystonia? There are further ways in which cerebellar function can be interrogated. For example I have not studied eye movements which are often one of the earliest signs of cerebellar pathology dysfunction. Furthermore the availability of automated eye trackers and the relative ease of collecting such data makes this approach attractive. Saccadic hypermetria, impaired saccadic adaptation, abnormal smooth pursuit (though this is poorly localising), and impaired vestibulo-ocular suppression can be considered oculomotor hallmarks of cerebellar dysfunction. As highlighted in the previous discussion we need to have certain criterion about how we rate such evidence in order to correctly interpret such data.

However the results of the thesis may suggest that such uni-dimensional approaches to exploring dystonia pathophysiology may be limited in their utility. We are defining dystonia as a network disorder yet repeatedly trying to test prescribed functions of single anatomical areas. The spectrum of genetic mutations causing dystonia are beginning to identify common cellular processes. Presumably a specific aspect of postural movement control has unique features that make it vulnerable to disturbance by the cellular processes such mutations confer. As such it may be more fruitful to interrogate network function despite the fact that such research is inherently more challenging.

Trials are already underway exploring stimulation of thalamic nuclei receiving cerebellar efferents in tremulous forms of dystonia. Very generally, the cerebellum is involved in pathophysiology of dystonia, is an anatomically accessible target and is a core communication site for sensorimotor control. It may be that these very broad statements alone support further therapeutic development of cerebellar stimulation techniques. As in the

case with pallidal stimulation, treatment evolution can advance despite limited understanding of precise mechanisms.

Dystonia is often described as an enigma and this remains a good analogy. The hope is that advances in analysis techniques and full integration of experimental findings across different research modalities will eventually offer us a more substantive insight into a disease which keeps its pathophysiology so hidden.

Appendix I: A reflection on plasticity research in writing dystonia

In recent years, attention has centered around the hypothesis of abnormal regulation of plasticity within sensorimotor circuits in isolated (primary) dystonia[77]. In theory, this hypothesis is very attractive. Increased plasticity in dystonia could result in an excessively responsive neuronal machinery with an increased tendency to form sensorimotor associations. Excessive plastic change and loss of selectivity resulting could slowly degrade motor control and lead to the clinical symptoms of dystonia. Genetic mutations that confer risk for dystonia could influence mechanisms that govern plasticity, and environmental risk factors, such as intensive practice in musicians' dystonia, can be eloquently incorporated into such an flexible mechanism [211]. Initial studies which shaped the plasticity hypothesis in dystonia used a version of paired associative stimulation (PAS) [212-216]. This method repeatedly pairs electrical stimulation of a peripheral nerve with transcranial magnetic stimulation (TMS) of the motor cortex [217]. The inter-stimulus interval is adjusted to ensure that inputs to the motor cortex initiated by nerve stimulation occur simultaneously with magnetic stimulation. It is widely accepted as a non-invasive manner in which to examine brain plasticity in humans [218].

In healthy controls, however, it is now appreciated that the response to plasticity paradigms such as PAS are highly variable between subjects. Some of the factors underlying this variability are beginning to be elucidated [120,218-220]. In fact, a large body of evidence has emerged since PAS was first described, such as the timing specificity and spatial focality, that has led to reinterpretation of many of the key features of PAS [221]. In the dystonia literature a similar pattern of increasing complexity has emerged. Early studies clearly described excessive effects of different PAS-protocols in focal hand dystonia [212,216]. However more recently, some studies failed to find any effect of PAS protocols in patients with focal dystonia [222], or no difference between the response of healthy subjects and those with dystonia [118]. In addition several papers now emphasise that the abnormality in dystonia may be subtler than a simple increase in plasticity in the target muscle group (often abductor pollicis brevis (APB), a median nerve innervated muscle). Instead, patients may have a greater spread of the effect to non-target muscles, such as abductor digiti minimi (ADM) (heterotopic spread), or a lack of homeostatic interaction between the response to PAS and other plasticity inducing protocols on the motor cortex [214,222].

In this work, we illustrate some of the features of PAS responses in dystonia by presenting data from 15 subjects with writing dystonia. We suggest that the variation in PAS response is

large, in keeping with that observed in neurophysiological studies of PAS in healthy subjects. We also review the existing literature examining PAS in writing dystonia.

Lack of a dystonic fingerprint in 15 patients with writing dystonia

As already noted, early studies examining plasticity responses in dystonia found excessive responses to plasticity protocols both in magnitude and spread to non-target muscles. Plasticity response is typically assessed by looking for increases in the mean amplitude of motor evoked potentials (MEPs), after PAS has been performed, as a surrogate marker of corticospinal excitability.

With this aim, we looked at the mean of 30 MEPs to target (APB) and non-target (first dorsal interosseous (FDI), ADM) muscles. The resting motor threshold (RMT), active motor threshold (AMT), and recruitment curves (RC), before (baseline) and after (at 0 min and 30 minutes (T0 and T30)) PAS, were also recorded. We used the archetypal variety of PAS in which the median nerve is stimulated 25ms prior to the TMS pulse to the motor cortex (PAS25) [97].

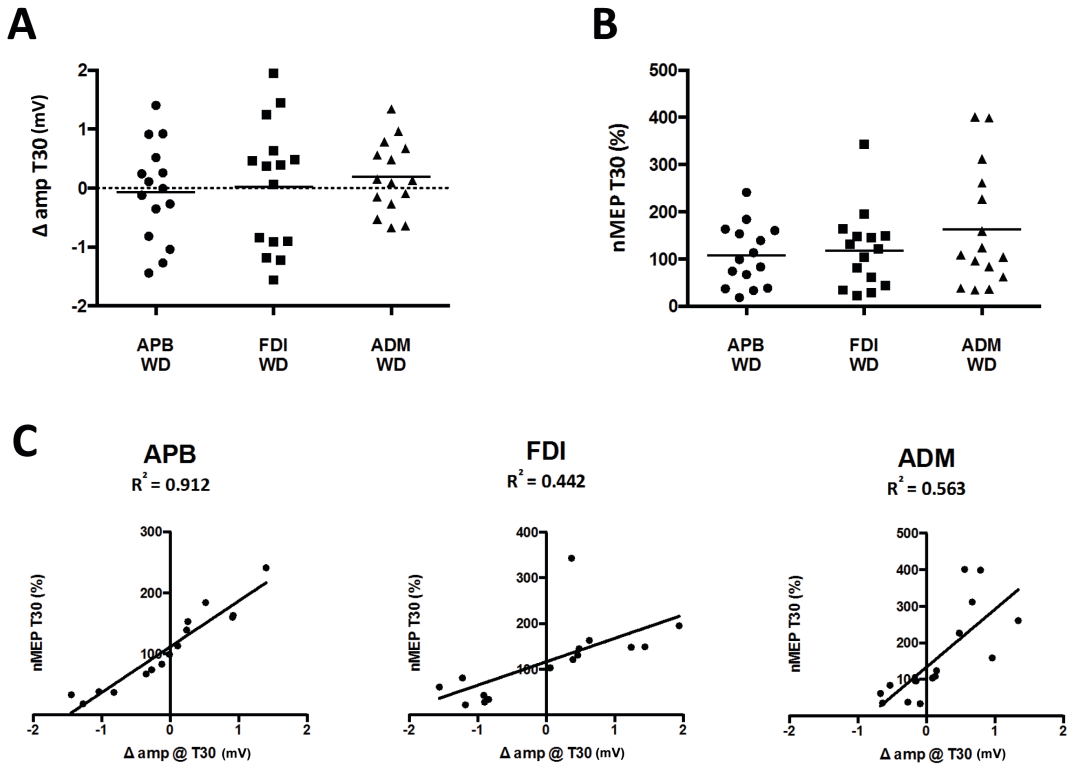


Figure A-1 Variability of PAS25 response *Each point is data from one subject. A Change in amplitude of 30MEP at 30 minutes (Δ amp T30) for the three hand muscles in writing dystonia demonstrating both inhibitors and facilitators to PAS25. B The same data as (A) shown as normalised MEP or % change to baseline (nMEP T30). This has been displayed in order to facilitate comparison with previous studies.* ***C*** *Correlation of nMEP and Δ MEP amp at T30. For APB (the motor hotspot) the shared variance is high (91.2%) however for FDI and ADM the shared variance is much lower*

(44.2% and 56.3% respectively). Both of these measures are used interchangeably currently in research articles.

Table A-1 and Figure A-1 gives an overview of the main results. If we first look at the 30MEP data it can be seen that there was no net change in the mean amplitude (mV) of the motor evoked potential in any of the intrinsic hand muscles tested. Thus, *no net* plasticity response was observed in APB, FDI or ADM, and 30MEP was remarkably stable at T0 and T30 in each muscle. In addition, baseline markers of corticospinal excitability, RMT and AMT, remained unchanged (given as % stimulus intensity (SI)). Furthermore, we looked in detail at the recruitment curves of each patient. The gradually increasing stimulus intensity used to elicit the RC should be sensitive to subtle shifts in corticospinal excitability outside the range of the '1mV' 30MEP stimulus intensity. We analysed the curves by fitting a linear regression to the RC (rRC) for *each* individual subjects for *each* muscle tested. There was no evidence for any change in motor cortex excitability as assessed by the slope of the rRC. In summary, plasticity was not excessive in magnitude or spread, in fact at a group level, very little PAS response was observed.

	Muscle	Baseline	T0	T30
RMT (%SI)	APB	44 ± 2.4	44 ± 2.5	44 ± 2.3
AMT (%SI)	APB	36 ± 2.2	36 ± 2.1	36 ± 2.1
30MEP (mV)	APB	1.1 ± 0.12	1.1 ± 0.12	1.1 ± 0.18
	FDI	2.1 ± 0.39	1.9 ± 0.29	2.1 ± 0.41
	ADM	1.2 ± 0.29	1.1 ± 0.27	1.4 ± 0.27
*r*RC (mV/%SI)	APB	1.2 ± 0.34	1.1 ± 0.30	1.2 ± 0.32
	FDI	1.6 ± 0.28	1.4 ± 0.26	1.5 ± 0.28
	ADM	1.1 ± 0.19	0.87 ± .17	1.2 ± 0.21

Table A-1 Minimal PAS25 response in 15 patients with writing dystonia. *Time points before (baseline) and after PAS25 (T0, T30). All data given as mean ± SEM, to 2 s.f. or nearest integer. RMT (F(2, 28)=0.84, p=0.44) and AMT (F(2, 28) = 1.1, p =.36) did not change over time. A significant effect of 'MUSCLE' on 30MEP was found (F(2,28) = 5.2, p = 0.012) but no change in 30MEP over 'TIME' (F (2,28)=0.68, p=0.52) or any significant interaction between 'MUSCLE*TIME' was seen (F(4,56)=0.78, p=0.54). There was no significant effect on rRC on 'MUSCLE' (F(2, 28) = 1.90, p = .16), 'TIME' (F(2, 28)=1.34, p=.278) or 'MUSCLE*TIME' (F(2, 28)=0.94, p=.447).*

Variability of PAS response

It is now known that in healthy subjects there is large inter-subject variability of the response to PAS. For example in a sample of twenty-seven people using a variety of PAS, only 14

showed the expected increase in corticospinal excitability, whereas the other thirteen exhibited a decrease[119,219]. Furthermore inter-individual variability of PAS response is indirectly acknowledged in studies that select PAS 'responders' (e.g. defining them as people who facilitate by at least 120%), and exclude those with no response or inhibition as 'non-responders' [116]. In addition, there may be individual day-to-day variation in the PAS response [219,223]. Our own data suggest that inter-subject variability to PAS is also likely to be an inherent feature of dystonia.

What underlies variability of PAS response?

To date genetic factors, cortical anatomy, age, gender, time of day, attention to paradigm, recent motor learning, life-long motor training, parallel motor activity, RMT, priming and pharmacological influences have all been shown to influence the magnitude of PAS response [119,224-229]. It is unlikely that routine experimental design can completely control for all of these and other yet to be identified factors. It is also possible that subtle differences in the way the PAS protocol is delivered (such as stimulus intensity, the number of pairs of stimulations and the rate of repetitions) may also affect outcomes.

The increased number of variables in a patient group such as dystonia, such as variability in phenotype and medications, are likely to complicate things further. We examined key clinical and electrophysiological parameters for their statistical power to predict PAS25 response in each muscle and did not find a clear relationship to the magnitude of PAS25 response (Table A-2).

		Clinical descriptors						Electrophysiological variables						
		Age (yrs)	Sex (M/F)	Previous botox (Y/N)	Duration of dystonia (yrs)	Overflow to other tasks	Presence of tremor (Y/N)	Baseline MEP	Δ RMT	Δ AMT	Δ ABP	Δ FDI	Δ ADM	Δ rRC
Statistical comparator		corr	t-test	t-test	corr	ANOVA	t-test	corr	corr	corr	corr	corr	corr	corr
PAS25 response	APB	.23	.98	.47	.97	.029	.65	.053	43	.16	-	.17	.34	.72
	FDI	.35	.87	.48	.47	.36	.64	.32	-	-	.17	-	.27	.09
	ADM	.87	.28	.29	.41	.44	.21	.14	-	-	.34	.27	-	.027

Table A-2 PAS25 responses in relation to clinical/electrophysiological parameters. *No clinical descriptors such as overflow of dystonia to other tasks or the presence of tremor demonstrated a clear relationship to PAS25 responses. No patients were taking medication known to influence PAS25 response. Individual electrophysiological variables also did not demonstrate potential to predict PAS25 response. For categorical clinical characteristics the p statistic is given from the independent t-test (binary categorical variable) or one-way ANOVA (nominal categorical variable). For continuous variables the p statistic (two-tailed) is given from Pearson's correlation. Muscle specific analysis was undertaken for baseline MEP and the change in rRC (i.e. change in the slope of the RC of ADM*

muscle from baseline to T30 was correlated with the change of amplitude of the MEP of ADM muscle from baseline to T30)

Such variability makes the interpretation of the pathophysiological significance of studies of PAS in dystonia rather difficult until the factors that can reliably predict PAS response are better understood. This variability could also explain the wide range of results observed in studies that attempt to replicate previous work (particularly if small numbers of subjects are used).

Review of PAS and writing dystonia

We performed a review of all studies that allowed data to be extracted for direct comparison and these are summarised in Figure A-2 and Table A-3. The overall impression is that the initial results have not been uniformly replicated in later work.

The first study on 10 patients found that there was an exaggerated response in the target muscle (APB: >300% facilitation) as well as facilitation of responses in non-target ("heterotopic") muscles that were normally unaffected by the median nerve PAS protocols (in this study FDI, although more commonly ADM is tested) [212]. Studies by Weise et al. and Belvisi et al. found a more modest exaggeration of the PAS response in writing dystonia, as well as excessive spread of the effect to heterotopic muscles [216,230]. However, Meunier and colleagues found *a smaller* response to PAS in patients with focal hand dystonia compared to healthy controls using a PAS protocol with a lower stimulus intensity (that evoked MEPs of 0.5mV at baseline) than in the "standard" protocol. When they increased the TMS intensity to evoke MEPs of 1mV in APB at baseline they did find facilitation in both APB and ADM (A), but responses did not significantly differ in magnitude from controls [118].

Interestingly, the fractional increase in the APB and ADM response following PAS were greater than in some other studies [216,230] that *did* find a difference in PAS response between writing dystonia and controls. This highlights the problem in defining abnormality with regards to a control group when variability is so high. A study by Hubsch et al., found a PAS25 response comparable to controls until a comparison at 30 minutes after PAS when the plasticity response was still present in patients with dystonia but not controls (in both APB and ADM muscles). Interestingly in the correlations of individual PAS responses detailed in this study one can also identify a significant proportion of patients with inhibition to PAS (as in our study). Finally, the present study and that of Kang *et al* demonstrated no overall effect of PAS[222].

Based on evidence from these studies it remains unclear whether the response in the target muscle, and indeed the non-target or heterotopic muscles, is consistently enhanced or not.

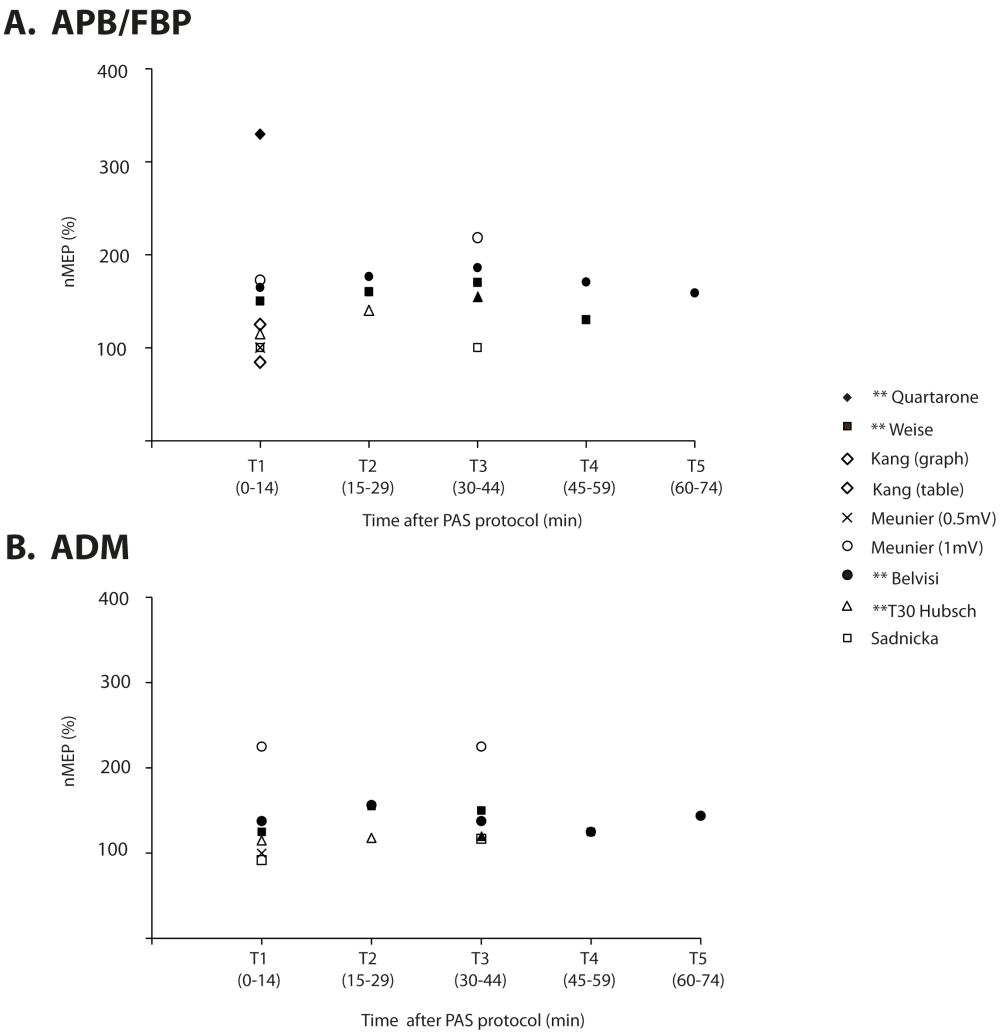


Figure A-2 Summary of previous studies

Mean nMEP for abductor or flexor pollicus brevis (APB/FPB) and abductor digitii minimi (ADM) are displayed. Studies are grouped into five time epochs (T1-T5) as detailed below the axis. The group mean is displayed as confidence intervals/standard error were not available all studies. Where a discrepancy between tabulated and graphical data was found both values are displayed. Table 3 accompanies this figure and gives the clinical details of patients and electrophysiological protocols used in each study. Studies which found a statistical difference between dystonic and control data are marked by a ***solid black*** *symbol (Quartarone, Weise, Belvisi, Hubsch at T30). The Meunier study (1mV) failed to find a significant difference between dystonia and control PAS responses yet had PAS responses greater than other studies that did find a difference between the two groups. This highlights the problem in defining abnormality with regards to a control group when variability is so high.*

First author	Year	Patients (n)	Mean age	Muscles recorded	Sensory stim to median n.	TMS M1 site	TMS SI %	Freq of pairs	No of pairs	ISI	Dur (min)	Conclusion
Quartarone	2003	Simple WD (10)	45	APB, FDI	100μs, 300%pT	APB	1mV	0.05Hz	90	25ms	30	Enhanced facilitation of APB and FDI muscles compared to controls.
Weise	2006	Simple and dystonic WD (10)	39	APB, ADM	200μs, 300%pT	APB	1mV	0.1Hz	180	21.5ms	30	LTP-like as well as LTD-like plasticity is abnormal with respect to magnitude, temporal properties and spatial organisation.
Kang	2011	Simple and dystonic WD (10)	47	APB	1ms, M-wave	APB	1mV	0.25Hz	225	25ms	15	No change in MEP amplitude seen after PAS25 in APB muscle.
Meunier (Exp 1) (Exp 3)	2012	WD and MD (13) (7)	51	FPB, ADM	250%pT	FPB	0.5 mV 1-1.5 mV	0.2 Hz	240	25ms	20	No difference in PAS25 response between patients and controls in either target FPB or non-target ADM in Exp 1 and 3.
Belvisi	2013	Simple and dystonia WD (10)	43	ABP, ADM	200μs 300%pT	APB	0.5-1mV	0.25Hz	200	25ms	13.3	Enhanced facilitation of APB and ADM muscles compared to controls
Hubsch	2013	Simple WD (21)	43	APB, ADM	250%pT	APB	90%AMT	5Hz	600	25ms	2	Comparable responses in APB and ADM in controls and dystonia until T30. At T30 facilitation only in dystonia group.
Sadnicka	-	Simple and dystonic WD (15)	54	APB, ADM	200μs, 300%pT	APB	1mV	0.2Hz	180	25ms	15	Group data reveals no PAS25 response in APB or ADM. Inter-subject variability highlighted.

Table A-3 Methodological details of previous studies examining PAS in writing dystonia *The first author and year the article was published index each study. 'Simple' WD describes patients in which dystonic features were present only when writing. 'Dystonic' WD is when dystonia also occurred with other manual activities. The number of patients (n) is bracketed. Muscles recorded included flexor pollicis brevis (FPB). Duration and intensity (as a percentage of the perceptual threshold (pT)) of the sensory stimulation to the median nerve (median n.) are given. The TMS hotspot (motor cortex (M1) site), and stimulus intensity (SI%) are described and the frequency and number of pairings, the interstimulus interval (ISI) and the total duration (Dur) of the PAS paradigm are detailed. No study has used the same PAS paradigm and the range of stimulation parametres is evident when studies are tabulated in this manner*

*M*ethodological observations

A methodological issue that can influence interpretation of the PAS response is the use of normalised MEPs (nMEPs). The nMEP is calculated by dividing the mean MEP post PAS by the mean baseline MEP. This gives a fractional change in magnitude and facilitates comparison between studies as it attempts to 'normalise' for variance in baseline MEP. One interesting repercussion of this calculation is that it may bias results in FDI and ADM. The variability of the baseline MEPs in FDI and ADM is probably greater than in APB as the motor "hotspot" is usually focused over the position that best elicits a reliable amplitude of MEP in APB. Thus if, as is often the case, the amplitude of MEPs recorded from ADM at baseline is very small, then a small increase in the magnitude of MEPs after PAS, will lead to a large percentage increase in facilitation (change in magnitude/small number x 100 = large percentage change). This is demonstrated graphically with our dataset: there is a weaker correlation between the effect of PAS calculated as nMEP and change in absolute MEP amplitude for FDI and ADM than in APB. There is also a larger range of percentage change values in FDI and ADM than in APB despite very similar changes in the absolute amplitude across the three muscles.

A further point regarding the nMEP is that by performing analysis in this way, inhibitors to PAS can only have a nMEP range between 0 to 100% while facilitators can have a range from 100% to infinity (the highest % change in nMEP in the current study was 401%). Taking an average of nMEP when there are both inhibitors and facilitators is therefore not valid mathematically as the range for facilitation is greater and thus mean data will tend to over-represent facilitation.

PAS in perspective

In health, as already emphasised there is considerable inter-individual and day-to-day variability in the response to PAS protocols. In addition, it has become evident that there is considerable complexity in the PAS effect itself [221]. For example, there is some evidence that multiple pathways may contribute to the PAS response rather than the most direct pathway as is often assumed [97]. Furthermore, PAS is no longer considered to be specific to the target muscle [221]. There are several reported instances in which changes in the excitability of corticospinal projections have been more pronounced in muscles innervated by a different nerve [231]. It is largely unknown what mechanisms of neuroplastic adaptation are engaged by PAS. Assumptions framing PAS as a method that evokes spike timing dependent plasticity at the synaptic level have been questioned and it is possible that a range of cellular mechanisms are involved, perhaps even at different levels of the motor system (for review see Carson[221]). Finally, abnormalities in PAS response have been demonstrated in a multitude of central nervous system disorders (for example: Alzheimer's disease [232], autism, [233], CADASIL [234], migraine [235], multiple sclerosis [236] and Parkinson's disease[237]). It remains a research challenge to define disease specific profiles of PAS response.

Conclusions

This viewpoint highlights the observation that some experimental evidence cannot be simply explained by the notion that there are consistently *exaggerated* responses to PAS25 in patients with writing dystonia. Whilst such a hypothesis remains valid, the variability of PAS is such that to date studies have been underpowered to answer this question. Irrespective of whether patients with dystonia show enhanced PAS25 responses, such an effect cannot be regarded as a "dystonic fingerprint" (at least for patients with writing dystonia) as the direction of response can vary and there is overlap between patient and healthy data. Furthermore, in healthy individuals PAS is no longer considered to be specific to the target muscle; arguments that dystonia has greater spread of response must also account for this finding in healthy subjects

Perhaps abnormal plasticity is not the primary driver of the clinical presentation. Clinically, one is struck by the highly conserved stereotypical abnormalities that are exhibited by each

patient. Although writing dystonia can spread to the other hand, it is typified by its stability over time and task specificity, which would not be clearly predicted from simple "runaway" plasticity. Similarly, loss of topographic specificity is not clearly supported by clinical cases as sometimes only an individual digit assumes the abnormal posture.

More generally, there are perhaps more questions than answers as to what PAS responses represent at the neuronal or synaptic level. Much work suggests that it cannot be assumed that PAS responses are a clear correlate to levels of synaptic plasticity and future research should try and define in a more specific manner what PAS responses signify in the dystonic brain.

Whilst seemingly disappointing, the conclusions drawn here may have important implications for the planning and outcome of future studies in this field. For example, it becomes difficult to use magnitude of PAS response at a group level as a marker of potential therapeutic effect of a novel intervention as it hides this individual variability and complexity. If individual plasticity profiles are given more weight within studies then subject-specific interventions may have greater potential. Otherwise at the group level a study which aims to 'reduce plasticity' may have its beneficial effects on excessive PAS responders hidden by a negative effect on those that have minimal response to PAS.

Our conclusions are limited to the use of PAS25 protocols in writing dystonia. We did not extend our review to other forms of non-invasive brain stimulation examining plasticity or those that assess the expression of homeostatic plasticity. These other protocols have been used in the same group of patients and some [214,215,238] but not all [230] have reported increased responses in dystonia. However, the same caveats may exist with this data. The numbers of patients examined with each protocol has been small. Given that the variation in response to theta burst protocols [120,239,240] and 1 Hz repetitive TMS [241] is at least as large as that to PAS, it seems likely that these effects may also fail to be replicated in some future studies.

Finally, we do not know if these conclusions are valid for other forms of dystonia. Other focal and generalised dystonias have also been reported to have increased responses to a variety of plasticity-inducing protocols [77,111,213,242-246]. Large multi-centre studies are needed to fully explore the variability of plasticity responses in these subtypes of dystonia and to better assess for potential clinico-neurophysiological correlations.

A limitation of this current work is that we have compared studies which have used slightly different PAS methods. As variations on methods have proliferated, deviations in results have become more numerous and these methodological variations are often held accountable. Methodological variation is not, however, the only explanation for the range of results observed in different studies using PAS paradigms. Indeed, scientific evaluation of

individual variability, which has its foundation in the physiology of each patient, may hold the key to defining the role of plasticity in the pathophysiology of dystonia.

Our work and that of others demonstrate unrecognised complexities regarding experimental methodology and pathophysiological assumptions in patients with writing dystonia. Better understanding of these factors is needed in order to advance the plasticity hypothesis in dystonia and to facilitate the search for novel treatments for this disabling condition.

Appendix II: Published papers arising from work during PhD period

Sadnicka A, Kassavetis P, Parees I, Meppelink AM, Butler K, Edwards M. Task-specific dystonia: pathophysiology and management. Journal of neurology, neurosurgery, and psychiatry. 2016.

Macerollo A, Chen JC, Parees I, **Sadnicka A**, Kassavetis P, Bhatia KP, et al. Abnormal movement-related suppression of sensory evoked potentials in upper limb dystonia. European journal of neurology : the official journal of the European Federation of Neurological Societies. 2016;23(3):562-8.

Saifee TA, Parees I, Kassavetis P, Kaski D, Bronstein AM, Rothwell JC, **Sadnicka A** et al. Tremor in Charcot-Marie-Tooth disease: No evidence of cerebellar dysfunction. Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology. 2015;126(9):1817-24.

Sadnicka A, Teo JT, Kojovic M, Parees I, Saifee TA, Kassavetis P, et al. All in the blink of an eye: new insight into cerebellar and brainstem function in DYT1 and DYT6 dystonia. European journal of neurology : the official journal of the European Federation of Neurological Societies. 2015;22(5):762-7.

Sadnicka A, Edwards MJ. The influence of reward and punishment on motor learning. Movement disorders : official journal of the Movement Disorder Society. 2015;30(13):1724.

Sadnicka A, Patani B, Saifee TA, Kassavetis P, Parees I, Korlipara P, et al. Normal motor adaptation in cervical dystonia: a fundamental cerebellar computation is intact. Cerebellum. 2014;13(5):558-67.

Sadnicka A, Hamada M, Bhatia KP, Rothwell JC, Edwards MJ. Cerebellar stimulation fails to modulate motor cortex plasticity in writing dystonia. Movement disorders : official journal of the Movement Disorder Society. 2014;29(10):1304-7.

Sadnicka A, Hamada M, Bhatia KP, Rothwell JC, Edwards MJ. A reflection on plasticity research in writing dystonia. Movement disorders : official journal of the Movement Disorder Society. 2014;29(8):980-7.

Popa T, Milani P, Richard A, Hubsch C, Brochard V, Tranchant C, **Sadnicka A** et al. The neurophysiological features of myoclonus-dystonia and differentiation from other dystonias. JAMA Neurol. 2014;71(5):612-9.

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Katschnig-Winter P, Schwingenschuh P, Davare M, **Sadnicka A**, Schmidt R, Rothwell JC, et al. Motor sequence learning and motor adaptation in primary cervical dystonia. Journal of clinical neuroscience : official journal of the Neurosurgical Society of Australasia. 2014;21(6):934-8.

Kassavetis P, **Sadnicka A**, Saifee TA, Belvisi D, van den Bos M, Parees I, et al. Motor 'surround inhibition' is not correlated with activity in surround muscles. The European journal of neuroscience. 2014;40(3):2541-7.

Grimaldi G, Argyropoulos GP, Boehringer A, Celnik P, Edwards MJ, Ferrucci R, **Sadnicka A** et al. Non-invasive cerebellar stimulation--a consensus paper. Cerebellum. 2014;13(1):121-38.

Sadnicka A, Kimmich O, Pisarek C, Ruge D, Galea J, Kassavetis P, et al. Pallidal stimulation for cervical dystonia does not correct abnormal temporal discrimination. Movement disorders : official journal of the Movement Disorder Society. 2013;28(13):1874-7.

Sadnicka A, Kassavetis P, Saifee TA, Parees I, Rothwell JC, Edwards MJ. Cerebellar transcranial direct current stimulation does not alter motor surround inhibition. The International journal of neuroscience. 2013;123(6):425-32.

Parees I, Saifee TA, Kojovic M, Kassavetis P, Rubio-Agusti I, **Sadnicka A**, et al. Functional (psychogenic) symptoms in Parkinson's disease. Movement disorders : official journal of the Movement Disorder Society. 2013;28(12):1622-7.

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