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Functional Imaging in Hereditary Dystonia

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Abbreviations: **BA**: Brodmann Area; **DRD**: dopa-responsive dystonia; **DTI**: diffusion tensor imaging; **FDG-PET**: fluorodeoxyglucose-PET; **^{18}F-DOPA**: fluorine-18-L-dihydroxyphenylalanine; **GABA**: gamma-aminobutyric acid; **GTP**: guanosine 5' triphosphate cyclohydrolase; **MAN-DYT1**: manifesting DYT1 mutation carriers, **MAN-DYT6**: manifesting DYT6 mutation carriers; **MRI**: magnetic resonance imaging; **NM-DYT1**: non-manifesting DYT1 mutation carriers, **NM-DYT6**: non-manifesting DYT6 mutation carriers,; **PET**: positron-emission-tomography; **PFC**: prefrontal cortex; **PreMC**: premotor cortex; **ROI**: region of interest; **SMA**: supplementary motor area; **SMC**: sensorimotor cortex; **SPM:** statistical parametric mapping.

SUMMARY

The development of causal treatments of dystonia is hampered by the lack of a clear etiological understanding of the disease. The classical model has conceptualized dystonia as a hyperkinetic basal ganglia disorder (Albin et al., 1989). However, neither post-mortem studies in primary dystonias nor animal models of dystonia have unequivocally confirmed this concept. Similarly, earlier imaging studies in primary dystonia have yielded highly conflicting results.

Thus, this series of neuroimaging studies set out to test the hypothesis of primary dystonia as a developmental sensorimotor neurocircuitry disorder affecting brain regions beyond the striatum. To focus on an etiologically most homogenous group, DYT1 dystonia was chosen as a potential prototype disorder of the primary dystonias. Additional studies were also conducted in the DYT6 and DYT13 genotype. These genotypes are not fully penetrant, offering the possibility to study effects of susceptibility and disease separately.

Using a multi-modal imaging approach, including positron-emission-tomography with the tracers $H_2{}^{15}O$, ^{18}F-FDG and ^{11}C-raclopride as well as magnetic resonance based diffusion tensor imaging, this series of studies supported the overarching hypotheses, showing that metabolic, microstructural and functional changes reach well beyond an isolated affection of the indirect striatal pathway, as suggested for hyperkinetic disorders traditionally (Albin et al., 1989). Similarly, a predominant role of dopaminergic neurotransmission within the basal ganglia was dismissed as hypothesized.

We demonstrated that functional changes of the striatum, both at the metabolic and the neurochemical level reflect the susceptibility for primary torsion dystonia (Carbon et al., 2004a, Carbon et al., 2009, Carbon et al., 2011; Carbon et al. 2013). By contrast, penetrance and disease severity did not relate to striatal characteristics, but were rather associated with the expression of a cortical sensorimotor brain activation network (Carbon et al., 2010). Nevertheless, downstream effects of striatal susceptibility-related changes in non-manifesting mutation carriers were found to manifest as sequence-learning deficits with concomitant cortical and cerebellar reorganization (Carbon 2004b, Carbon et al. 2008b, Carbon et al. 2011). Importantly, microstructural abnormalities underlying the expression of cortical sensorimotor network changes were found in the cerebellar outflow pathways in dystonia mutation carriers (Carbon et al. 2008a; Argyelan/Carbon et al., 2009). Interestingly, we found a novel interaction of cerebello-thalamic and thalamo-cortical connectivity as a potential mechanism regulating penetrance in the DYT1 and DYT6 genotype (Argyelan/Carbon et al., 2009). Thus, our data suggest that the functional entity of sensorimotor circuitry rather than one node within this network regulates symptom expression in dystonia.

A. INTRODUCTION

Dystonia is a neurological syndrome that has classically been defined by “sustained muscle contractions, frequently causing twisting and repetitive movements or abnormal postures” (Marsden et al., 1987). This definition has recently been expanded to “a movement disorder characterized by sustained or intermittent muscle contractions causing abnormal, often repetitive, movements, postures, or both. Dystonic movements are typically patterned, twisting, and may be tremulous. Dystonia is often initiated or worsened by voluntary action and associated with overflow muscle activation.” (Albanese et al., 2013).

Dystonia varies highly in clinical severity, but even in focal variants the quality of life is markedly reduced (Müller et al., 2002). Due to the lack of clear etiological understanding of the various dystonias, to date, all treatment options of dystonia are merely symptomatic (Jankovic, 2009). Importantly, a clearer understanding of mechanisms of disease would be needed for the development of causal therapies for dystonias of various etiologies. Thus, the objective of the following series of studies was a contribution to our understanding of mechanisms of disease in dystonia. This background section aims to provide a summary of the existing the body of knowledge on pathophysiology of dystonia, and of the current contribution of imaging studies to these concepts.

A.1. Classification of the Dystonias

A.1.a. Clinical Classification

Clinically, dystonia occurs in a vast variety of contexts, thus classifications of the dystonias are a topic of continued discussion (de Carvalho and Ozelius, 2002; Bressman, 2004; Albanese et al., 2013). The current consensus classification scheme categorizes the different dystonias by clinical characteristics (with sub-classification per age at onset, body distribution, temporal patterns and presence or absence of additional neurological features) and by etiological characteristics (with sub-classification by presence or absence of nervous system degenerative or macroscopic structural pathology and acquired vs. inherited etiology; Albanese et al., 2013). Importantly, despite these classification attempts, dystonias show considerable phenotypic variability with a huge overlap among different syndromes and etiologies, and at the same time a lack of consistent clinical-etiological correlations, both for genetic and for acquired causes.

For research, grouping strategies guided by current knowledge on biological mechanisms may be superior to clinical classification systems. Thus, in order to recruit cohorts with presumably most homogenous mechanisms of disease, genetically defined subgroups of primary dystonia (DYT1, DYT6, DYT11) were studied in this line of research. Of note, these cohorts were clinically mixed with regard to the body distribution of dystonia, thus minimizing the confounding effect of specific abnormal movement patterns.

A.1.b. Genetic Classification

There are currently more than 20 genetic loci that have been associated with monogenic dystonias (Petrucci and Valente 2013). These can be grouped into the three major clusters, of isolated dystonia (including the DYT1 and DYT6 genotype amongst others), dystonia plus syndromes (e.g. DRD, DYT11) and paroxysmal dystonias. Among these, the gene product is known for DYT1 (gene coding for torsinA), DYT6 (gene coding for THAP1), DRD (gene coding for GTP-Cyclohydroxlase/GTPCH1) and DYT11 (gene coding for epsilon sacroglycan); and most recently for DYT4 (gene coding for β- tubulin 4a; Hersheson et al., 2013).

However, the functions of the different gene products in health and disease are still a topic of current research, and a common pathway for these proteins does not seem to evolve. To date, genetic studies do not converge on the involvement of a specific neurotransmitter system or another common pathway to be involved in the dystonias, nor have they implied a common brain region predominantly involved in the dystonias. Rather the presence of numerous genotypes without clear genotype-phenotype association invites the speculation that multiple brain regions and/or neurotransmitter systems can be involved in the various forms of dystonias. In this context, the subsequent series of studies was designed to deepen our understanding of hereditary dystonias, assuming that these monogenic diseases might serve as prototype disorders and that mechanisms of disease identified in one disorder would provide testable hypotheses for the more frequent, but yet even more heterogeneous sporadic dystonias. Thus, the most frequent hereditary dystonia, DYT1 dystonia, was studied as a prototype disorder to help identify mechanisms of disease that could be translated to sporadic dystonias in the future.

DYT1 dystonia is clinically characterized by an early onset, often starting in one limb (leg) and spreading during the prepubertal period throughout the body. In addition to this typical course of ultimately generalized dystonia, DYT1 dystonia can also manifest as isolated task-related or simple focal dystonia. DYT1 dystonia is inherited as an autosomal dominant DYT1 mutation on gene 9q34 representing a GAG deletion within the coding area for torsinA (Ozelius et al., 1997). This deletion, however, causes clinical manifestations of dystonia in only 30% of mutation carriers (Bressman et al., 1994).

DYT6 dystonia represents another less frequent autosomal dominant variant of primary torsion dystonia, which has been characterized in North America Mennonites (Almasy et al., 1997). DYT6 had been linked to a mutation on chromosome 8 (8p11-8q13), but had not yet been fully identified at the beginning of our studies. In 2009, the DYT6 mutation was identified as a missense mutation of the THAP1 gene (Fuchs et al., 2009). Clear factors determining penetrance have not been identified in both genotypes.

DYT 11 represents the most frequent genetic variant of myoclonus dystonia and has been related to various loss of function mutations in the epsilon-sarcoglycan gene (SGCE) located on chromosome 7q21 (Zimprich et al., 2001, Valente et al., 2005).

A.2. Pathophysiology of Dystonia

Dystonia has traditionally been conceptualized as hyperkinetic basal ganglia disorder (Vitek et al., 1999). This pathophysiological concept has been derived from lesion studies of secondary dystonias, involving the basal ganglia (Bhatia and Marsden 1994) and from several lines of evidence that strongly suggested a key function of the dopaminergic system in dystonia (Hornykiewicz et al., 1986; Ichinose et al., 1999; Wichmann, 2008). However, a seminal cohort study on secondary dystonia disclosed an important contribution of the cerebellum and its afferents (Ledoux and Brady 2003). Currently, mounting evidence confirms a prominent role of these regions in conjunction with sensorimotor circuitries (Eidelberg et al., 1998; Tanabe et al., 2009; Neychev et al., 2009).

In the following section, the contributions of different research techniques to our understanding on pathophysiology of dystonia will be summarized. Notably, this body of knowledge has continuously broadened from the traditional view of dystonia as a basal ganglia disorder throughout the period of conduction and publication of the studies summarized subsequently. Thus, parallel gains of knowledge from fields, other than imaging, will be summarized in the introduction, even though some of them had not yet been detected at the time of our studies. The introductory section regarding imaging was restricted to knowledge up to 2004 as this reflects the starting point of this line of work; the subsequent evolution is discussed in the context of the studies and the summarizing discussion.

A.2.a. Human Morphological Studies

The earliest postmortem study on idiopathic childhood onset generalized dystonia (n=2), reported a lack of histopathological abnormalities, normal levels of acetylcholine, GABA and glutamate, but reduced dopamine levels in the striatum in one subject and reduced levels of norepinephrine in the subthalamic nucleus, the hypothalamus, the mammillary bodies and locus ceruleus in both subjects (Hornykiewicz et al., 1986). However, subsequent postmortem studies in sporadic primary dystonias have failed to provide consistent findings (for review see Standaert, 2011). Similarly, postmortem studies in DYT1 dystonia have yielded inconsistent results with normal regional distribution of torsinA in the adult brains (Walker et al., 2002; Rostasy et al., 2003), but modest changes in dopamine metabolites suggestive of altered dopamine turnover (Furukawa et al., 2000; Augood et al., 2002). The most detailed study in four DYT1 dystonia autopsy cases revealed ubiquitin-torsinA-positive inclusions in brainstem regions, including neurons of the pedunculopontine tegmental

nucleus, the cuneiform nucleus, and periaqueductal gray matter, but not in striatal or cortical regions (McNaught et al., 2004). Importantly also no association with the dopaminergic system was found, instead the inclusions co-localized with cholinergic markers (McNaught et al., 2004).

A.2.b. Animal Models of Dystonia

Similarly, results from animal models of dystonia have not led to a conclusive understanding of the disorder yet, because to date there is no model that combines a clear etiology with a clear dystonic phenomenology (LeDoux, 2011; Jinnah et al., 2009). Phenomenological and etiological rodent models, as well as - to a lesser degree - primate models contribute different aspects to considerations on potential mechanisms of disease in humans.

A.2.b.i. Phenomenological Models

Phenomenological models show abnormal movement patterns reminiscent of human dystonia, which were serendipitously detected as natural variants in breeding colonies. These include the spontaneously dystonic *dt* rat (Lorden et al., 1984), the tottering mouse (Green and Sidman, 1962), and the dystonic (*sz/dt*) hamster (Nobrega et al., 1995). While the *dt* rat is considered closest to primary dystonia, both the sz/dt hamster and the tottering mouse reflect paroxysmal dystonia. Moreover, the attacks of the tottering mouse are highly stereotyped beginning in the lower extremities and further expanding to the upper extremities and the trunk. Unlike humans with primary dystonia, however, tottering mice and the sz/dt hamster have normal motor abilities between the attacks.

Even though the *dt* rat is clinically severely affected with postnatal progressive axial and appendicular dystonia leading consistently to death, no obvious abnormalities of brain morphology were found (Lorden et al., 1984, LeDoux et al., 1995) and extensive studies of basal ganglia morphology and neurochemistry similarly failed to identify abnormalities (Mc Keon et al., 1984). A subsequent series of studies regarding cerebellar morphology in the *dt* rat revealed normal Purkinje cell number and arborization, normal volume of the cerebellar nuclei, normal soma size of cerebellar nuclear neurons, normal molecular and granular cell layer thickness and normal cerebello-olivary connectivity patterns. Only the size of Purkinje cells of the vermis and paravermis at postnatal day 20 was slightly reduced (5–11%; Lorden et al., 1992). Despite the paucity of morphological abnormalities, markers of functional activity of the cerebellar cortex were found to be abnormal: Spontaneous Purkinje cell firing rates were reduced (Stratton et al., 1988), cyclic guanosine monophosphate (cGMP) was found to be decreased in rat cerebellar cortex (Lorden et al., 1985), and the activation response to chemically induced activation or electrical activation was found to be attenuated (Stratton et al., 1988; Lorden et al., 1985). These changes were suggested to relate to

increased activity of GABAergic projections of Purkinje cells onto deep cerebellar nuclei (LeDoux, 2011). Indeed, the crucial role of the cerebellum in the *dt* rat was proven in experiments where cerebellectomy prior to the expression of the dystonic syndrome prevented the development hereof and led to survival past adulthood (LeDoux et al., 1993). Notably though, with regard to etiological aspects this animal model clearly differs from human causes of primary dystonia: the mutation responsible for this *dt* rat was localized to the ATCAY gene (Xiao and Ledoux, 2005), but mutations within the human ATCAY gene lead to a syndrome of combined mental retardation, gait ataxia, intention tremor, nystagmus and dysarthria, but not to dystonia (Bomar et al., 2003).

A.2.b.ii. Etiological Models - Genetic

Among etiological animal models genetically engineered mouse models are most relevant to the subsequently summarized line of work in humans. Unfortunately however, all transgenic mouse models with heterozygous expression of the human DYT1 mutation failed to replicate motor abnormalities clearly reminiscent of human dystonia or interfering with breeding and survival (cf. Jinnah et al. 2008). Considering the 30% penetrance for the DYT1 mutation in humans (Bressman et al. 1994), it would not be surprising to find only certain subgroups of transgenic animals to be affected, however a clear clustering of motor abnormalities has not been reported in any of the transgenic models. Nevertheless, even in the absence of overt dystonia, certain pathophysiological aspects thought to be important in human dystonia were found in some transgenic models, albeit with very moderate impact: Increased striatal DA turnover appears to be the most consistent finding, demonstrated in three models, in which the observed motor abnormalities consisted of self-clasping behavior during the tail suspension test (Shashidharan et al., 2005), decreased dexterity during beam walking (Zhao, 2008) and impaired motor learning skills (Balcioglu et al., 2007). In addition, a mutant DYT1 knock-in mouse model showed minimal motor abnormalities upon extensive testing and slight reductions in striatal dopamine in male mice, but not in females (Dang et al., 2005) and a torsinA knock-down mouse model reported slightly abnormal open field activity and dexterity in beam walking in male mice, but not in females along with slightly reduced metabolites of dopamine (Dang et al., 2006).

A.2.b.iii. Etiological Models – Induced Lesions

Analogous to lesion locations identified in secondary dystonias, animal models using direct surgical or chemical lesion techniques were introduced. Indeed, using these techniques (in contrast to genetic modeling), overt dystonia could be convincingly reproduced in mammals. However, in these models the relationship to idiopathic dystonia, which is the most frequent type in humans and which lacks clear lesional findings, may be rather limited. The value of

these models, however, lies in providing testable hypotheses for mechanisms of disease in idiopathic dystonia and in providing models for pharmacological testing.

Generalized and segmental dystonia has been modeled in rodents with chemical lesions of the basal ganglia (Guyot et al., 1997), the cerebellum (Pizoli et al., 2002) and the brainstem red nucleus (Walker et al., 1988). Interestingly, a line of early research has demonstrated the induction of (mostly transient) cervical dystonia with lesions of the ventral tegmental area, including the interstitial nucleus of Cajal (Foltz et al., 1959, Battista et al., 1976, Sambrook et al., 1979, Mori et al., 1985). Though the ventral tegmental area mainly contains dopaminergic neurons, there are serotonergic neurons close to its border, and the torticollis inducing lesions were neurochemically associated with a reduction of serotonin in the caudate nucleus (Miyake, 1988).

Models of maladaptive sensorimotor integration have been used for other focal dystonias. Of particular interest was the blepharospasm model developed by Schicatano and colleagues (1997), as this two-hit model may be close to the situation in humans, where the specific combination of subthreshold internal and external factors results in the manifestation of symptoms. In this model, an incomplete brainstem dopamine depletion (as can be present in aging) and an incomplete denervation of the orbicularis oculi muscle (representative of an unspecific ocular irritation) led to the manifestation of blepharospasm in the rat.

Another important model of focal dystonia closely parallels task induced dystonias, as it represents consequences of strenuous overuse (Byl et al. 1996), emphasizing the environmental aspect over the internal one. In this model affected primates, who had been trained to perform highly stereotyped, repetitive hand movements, show dedifferentiated somatosensory cortices, relating to the observation of increased cortical neuronal plasticity, discussed for humans below.

A.2.c. Neurophysiological Studies of Dystonia in Humans

Electrophysiological studies have consistently shown impaired inhibition, increased neuronal plasticity, and/or abnormal sensory processing in dystonias of various clinical subtypes and origins (Hallett, 2011). Inhibitory loss has been demonstrated at key levels of the central nervous system, i.e. the spinal, brainstem and cortical level (Hallett, 2011). Loss of inhibitory cortical function can be considered the neurophysiological hallmark of dystonia, but exactly how this common downstream pathway is generated remains a topic of current research, where dysfunction of the basal ganglia, the brainstem and the cerebellum alone or combined or interrelated are being discussed. Classically, cortical dysinhibition has been explained as a failure of cortico-striato-pallidal-thalamo-cortical circuit (CSPTC) function; i.e. involuntary movements were interpreted as a failure to select the appropriate movement pattern and to inhibit non-selected “overflow” movement”. In this regard, frequency reductions in pallidal

output have been recorded in vivo in humans with dystonia (Silberstein et al., 2003; Starr et al., 2005; Nambu et al., 2011; Nishibayashi et al., 2011), and in DYT1 transgenic mice (Chiken et al., 2008). The finding of impaired cortical inhibition was corroborated in transcranial magnetic stimulation (TMS) studies (Quartarone et al., 2006; Rothwell, 2007; Di Lazzaro et al., 2009), which showed both, the loss of short and long intracortical inhibition as well as a decreased silent period. While these studies clearly capture the pathophysiology of the dystonic movement as such, the etiological role of these cortical changes could not be identified, particularly, considering that neither lesion studies, nor postmortem histopathology, nor animal models have isolated the potential pathology to cortical structures.

Abnormally reduced pallidal firing frequencies have directly been measured in patients undergoing surgery for deep brain stimulation for primary dystonia (Silberstein et al., 2003; Starr et al., 2005; Weinberger et al., 2012). Similarly, dystonia related to dopamine depletion was linked to low pallidal frequencies (Kühn et al., 2008). By contrast, in a single patient with a striatal stroke, however increased rates of bursting pallidal neurons were found (Fuller et al. 2013). Subthalamic nucleus rates have been found to range higher than normal in primary adult onset dystonia (Schrock et al. 2009). Close correlations of subthalamic neuronal activity (Zhuang et al., 2004; Neumann et al. 2012) or pallidal neuronal activity (Chen et al. 2006) and dystonia-related EMG activity were observed in primary and secondary dystonia.

Closely related to the loss of inhibition at several levels is the observation of increased neuronal plasticity in dystonia (Classen, 2003; Siebner et al., 2003; Quartarone et al., 2006). Along these lines, magnetencephalography showed altered cortical representation patterns, with reduced distances of motor and sensory representations of single digits of dystonic hands (Elbert et al., 1998; Braun et al., 2003).
In summary the plethora of neurophysiological studies of various forms of dystonia clusters around the observation of impaired neuronal inhibitory control at various levels and with widespread consequences. The identification of the origins of these abnormalities has however been beyond the scope of neurophysiology.

A.2.d. Imaging Studies of Human Dystonia

Compared to neurophysiological techniques, which typically target one functional system at a time, neuroimaging techniques offer a non-invasive in vivo whole-brain overview of specific tissue characteristics or of functional reactivity (depending on the method used). On one hand this broadens the scope of the measurements on the other hand causal inferences of observations are clearly limited.

Despite the promising nature of neuroimaging, imaging studies of dystonia have yielded highly conflicting results, which is in stark contrast to the rather consistent findings of neurophysiological studies. Differences in specific techniques, study design and analytical techniques likely contribute to the inconsistencies. It is possible though, that the heterogeneity also reflects the true variety in mechanisms of disease (identified in imaging), which in turn lead to a common downstream pathway (identified in neurophysiology).

As mentioned above, this introductory chapter will be restricted to the background of neuroimaging studies prior to the start of the subsequently summarized series of studies in hereditary dystonia.

A.2.d.i. MRI Based Studies of Dystonia

Voxel-based morphometry (VBM): VBM as a basic structural technique provides data of regional brain volumes using T1-weigthed MRI scans. Using this technique, volumetric increases of basal ganglia structures were shown in patients with blepharospasm or focal hand dystonia (Black et al., 1998), in cervical dystonia (Draganski et al., 2003) and in DYT1 dystonia (Draganski et al., 2009). Volumetric increases were noted in cervical dystonia in the SMA, in the PFC, and in the visual cortex (Draganski et al., 2003) and in the primary sensory cortex and in the primary motor cortex in focal hand dystonia (Garraux et al., 2004).

Functional MRI (f-MRI): Up to 2004, there were only a few functional MRI studies due to the limited availability at that time and all of them only included subjects with various focal dystonia (as opposed to the clinically mixed series of hereditary dystonia summarized here). Studies using motor paradigms devoid of inducing overt dystonia during imaging in focal hand dystonia found decreased SMC and SMA activation during contraction and relaxation without concomitant activation increases (Oga et al., 2002) and increased, persistent basal ganglia, primary motor cortex and sensory motor cortex activation without concomitant activation decreases (Blood et al., 2004). In addition, other f-MRI studies in focal hand dystonias using motor paradigms with dystonic movement during the scanning found increased primary motor cortex activation (Preibisch et al., 2001; Pujol et al., 2000) and increased cerebellar activation (Preibisch 2001), as well as decreased premotor cortex activation (Pujol et al., 2000). In blepharospasm, abnormal putaminal activation was recorded selectively during periods of the spasm (Schmidt et al., 2003).

f-MRI studies using sensory paradigms showed altered processing of simultaneous inputs (Sanger et al., 2002; Butterworth et al., 2003, Delmaire et al. 2005) and increased basal ganglia activation (Peller et al. 2006) In parallel to the findings of the primate overuse model of dystonia, altered somatotopy was found with reduced distances of cortical representations of single digits during vibro-tactile stimulation (Butterworth et al., 2003).

The heterogeneity of functional activation studies, despite comparable disease cohorts, may result from differences in (1) activation paradigms, (2) baseline states, and (3) analytical techniques as well as from the fact that these studies typically involve rather low numbers of subjects due to the difficulty to recruit affected subjects and due to the necessity to perform these studies during a tight time frame to avoid drift in scanner measures.

MR based spectroscopy: Consistent with the observation of reduced inhibition at many levels of the central nervous system, one small study using MR based spectroscopy of GABA metabolites in writer's cramp (n=7) reported significant reductions in the GABA levels of the SMC and lentiform nuclei contralateral to the affected hand (Levy and Hallett 2002). A later, larger, confirmatory study (n=22) by the same workgroup in a similar cohort (task specific hand dystonia), however, failed to reproduce this observation (Herath et al., 2010).

A.2.d.ii. Positron-Emission-Tomography Studies of Dystonia

Activation Studies: Motor activation studies using radiolabeled water or oxygen and PET were conducted, contrary to the fMRI studies, in cohorts of focal dystonia and in segmental or generalized dystonia. A series of studies by Ceballos-Baumann and colleagues showed increased activation of the premotor cortex (PreMC) and SMA in acquired hemidystonia (Ceballos-Baumann et al., 1995a) and in idiopathic torsion dystonia (Ceballos-Baumann et al., 1995b). However, putaminal overactivation was only found in idiopathic torsion dystonia (Ceballos-Baumann et al., 1995b), whereas cerebellar overactivation was only found in acquired hemidystonia (Ceballos-Baumann et al., 1995a). Moreover, contrary to the f-MRI studies, *de*creases in primary motor cortex studies were reported in his studies (Ceballos-Baumann et al., 1995a,b; 1997), as well as in another seminal study of idiopathic torsion dystonia (Playford et al., 1998). In the latter study, similar to the first studies by Ceballos-Baumann, increases in PreMC, SMA, cerebellum and putamen were reported. By contrast, the three studies (up to 2004) in writer's cramp showed opposing results, reporting *de*creased PreMC and primary motor cortex activation (Odergreen et al., 1998) but also *in*creased PreMC (Lerner et al.; 2004; Ibanez et al., 1999) and primary motor cortex activation (Ibanez et al.; 1999). Yet different results were reported for dorsolateral-prefrontal cortex (DLPFC) activation during motor tasks, where abnormal overactivation was seen in patients with primary generalized dystonia with deep brain stimulation during the OFF period with a normalization during the ON period (Detante et al., 2004). Similarly, another PET study in a single patient with GPi DBS (Kumar et al., 1999) characterized the effect of stimulation as reduced activation in the primary motor cortex, PreMC, SMA and DLPFC.

The heterogeneity of results was however less apparent in the few studies focusing on sensory paradigms, which confirmed the observation of abnormal sensorimotor integration in dystonia (Tempel and Perlmutter 1990; Feiwell et al., 1999).

Resting state studies: Considering that motor activation paradigms may be subject to a variety of confounds in dystonia, PET studies into glucose metabolism in dystonia were expected to yield more consistent results. PET studies using radiolabeled glucose in focal dystonia had been conducted in blepharospasm mainly (Esmaeli-Gutstein et al., 1999; Hutchinson et al., 2000; Kerrison et al. 2003), but also in cervical dystonia (Magyar-Lehmann et al. 1997). Three of these found increased metabolism in basal ganglia circuits, however all in varying topographies, i.e. in the striatum (Magyar-Lehmann et al., 1997; Esmaeli-Gutstein et al 1999) in the caudate (Kerrison et al., 2003) and in the thalamus (Esmaeli-Gutstein et al., 1999). It has to be noted though, that some of these earlier studies accepted significance thresholds that would no longer be considered significant by current standards. Contrasting to the other studies, only one found pontine and cerebellar hypermetabolism (Hutchinson et al., 2000).

In a series of PET studies using FDG and spatial covariance analysis (Alexander and Moeller 1994) an abnormal idiopathic torsion dystonia related network was identified (Eidelberg et al., 1995). This network showed relative hypermetabolism of prefrontal and paracentral regions in conjunction with striatum, pons and midbrain. Interestingly, the intensity of this network correlated with the clinical severity of dystonia. After the identification of the DYT1 genotype (Ozelius et al. 1997) a DYT1-specific metabolic brain network was derived from scans in NM-DYT1. This network was characterized by relative increases in the posterior putamen, cerebellum and SMA (Eidelberg et al., 1998). Importantly, abnormal DYT1-specific metabolic network activity was demonstrated in symptomatic DYT1 carriers when involuntary dystonic movements were suppressed by sleep induction (Eidelberg et al., 1998). This ROI-based network was subsequently confirmed using voxel-based analyses (Trost et al., 2002). The extension of this work into the DYT6 genotype is one of starting points of work presented later (cf. Chapter B.1).

Neurotransmitter studies: A unique quality of PET is the ability to analyze neurotransmitter profiles. Ample evidence suggests a role of dopaminergic neurotransmission in symptoms of dystonia; particularly the clinical observations of DRD, antipsychotic induced acute or tardive dystonia (e.g., Burke et al., 1982; Factor and Matthews, 1991; Casey, 2004). Moreover, a number of secondary dystonias appear to be solely caused by abnormalities of dopaminergic transmission, as has been documented by PET studies (Ernst et al., 1996; Wong et al., 1996; Brashear et al., 1999, Asanuma et al., 2005).

PET studies have reported abnormalities of dopaminergic neurotransmission with reduced striatal D_2 receptor availability in cervical dystonia (Hierholzer et al.; 1994, Naumann et al.; 1998), in focal hand or cranial dystonia (Perlmutter et al., 1997). Notably, in the most severely affected cases, in generalized idiopathic torsion dystonia, ^{18}F-DOPA uptake, as a correlate of dopamine decarboxylase activity was only slightly reduced in the majority of

dystonic patients studied, with only three of eleven subjects showing values clearly outside the normal range (Playford et al., 1993).

A.3. Aims and Hypotheses

The overview on the body of knowledge on pathophysiology in dystonia (particularly at the time of the start of these projects) illustrates that a deep understanding of the disease is clearly lacking, even though this is sorely needed to develop treatment strategies beyond mere symptomatic treatment. In particular, the data from human post-mortem, neurophysiological and imaging studies in conjunction with those from animal models question the simplistic model of dystonia as a hyperkinetic basal ganglia disorder. Abnormalities on the cortical level, the basal ganglia level, the brainstem level and the cerebellar level were convincingly and repeatedly shown in different studies, and thus needed to be integrated. A contribution to the development of an integral model was the ultimate aim of this series of studies.

It was and is uncertain though whether or not the dystonias are unified by a common mechanism of disease, or whether multiple mechanisms can lead to a similar clinical manifestation or to a shared downstream pathway. To study cohorts with a high likelihood of a unifying mechanism of disease, we chose to study hereditary dystonias. In particular DYT1 dystonia was a focus, as this mutation was identified as of 1997 as an autosomal dominant DYT1 mutation on gene 9q34 representing a GAG deletion within the coding area for torsinA (Ozelius et al.,1997). The studies were then expanded into carriers of the DYT6 gentoype, even though at the time the studies the gene had not yet been identified.

The overarching hypotheses tested in the subsequent studies were:

1. Based on earlier imaging studies (Eidelberg et al., 1995; Trost et al., 2002), we hypothesized that *hereditary dystonia is associated with a network of functional changes in primary and secondary motor cortices, in conjunction with changes in basal ganglia and cerebellar structures* rather than with isolated dysfunction of the indirect striatal pathway as posited earlier (Albin and Young, 1989). This hypothesis was tested:
    a. in manifesting and non-manifesting DYT1, DYT6 and DYT 11 mutation carriers using voxel-wise analyses of brain glucose metabolism as measured in FDG PET (**B.1.**: Carbon et al., 2004a; **B.2.**: Carbon et al., 2013),
    b. in manifesting and non-manifesting DYT1 and DYT6 mutation carriers using using voxel-wise analyses of microstructural connectivity data as measured with DTI-MRI scans (**B.3.:** Carbon et al., 2004b; **B.4.:** Carbon et al., 2008a; **B.5.:** Argyelan/Carbon et al., 2009) and

c. in manifesting and non-manifesting DYT1 and DYT6 mutation carriers using using voxel-wise analyses of D_2 receptor binding as measured in raclopride PET (**B.6.:** Carbon, 2009a).

2. Based on our findings in the resting state, we hypothesized that a complex interaction of network changes mediates cortical activation changes in genetically prone individuals. This hypothesis was tested:
   a. in manifesting and non-manifesting DYT1 mutation carriers using voxel-wise analyses of brain activation $H_2{}^{15}O$-PET studies during simple paced motor tasks (**B.7.:** Carbon et al., 2010a). [Extending data of a small cohort of subjects with sporadic dystonia and with deep brain stimulation will also be included.]
   b. in manifesting and non-manifesting DYT1 mutation carriers using voxel-wise analyses of brain activation $H_2{}^{15}O$-PET studies during motor sequence learning (**B.10.:** Carbon et al. Brain 2008b; **B.11.:** Carbon et al. 2011).

To date, the entire series of studies involved 25 manifesting (MAN) and 18 non-manifesting (NM) DYT1 mutation carriers, and 11 MAN-DYT6 and 13 NM-DYT6 mutation carriers. The majority of these subjects were scanned in the resting state with ^{18}F-FDG PET. Different subgroups were additionally imaged with: (1) ^{15}O-water ($H_2{}^{15}O$) PET to assess the effects of genotype and phenotype on regional activation responses during motor performance and sequence learning; (2) MRI-DTI to assess corresponding changes in anatomical connectivity as well as (3) ^{11}C-raclopride to examine the role of striatal D_2-receptor binding in mediating these functional/anatomical changes. A separate cohort with myoclonus dystonia (DYT11) was scanned with ^{18}F-FDG PET. Moreover, ongoing studies have included subjects with sporadic dystonia as well as subjects with deep brain stimulation in ON/OFF states. Additional pilot studies have transferred the PET studies to f-MRI technique.

The prime methodological advantage of our studies is the inclusion of clinically unaffected, non-manifesting mutation carriers. In this regard, we have taken care not to recruit preclinical subjects as non-manifesting subjects. This is possible due to the typically early age of manifestation, around the age of 8 years in DYT1 dystonia, and around puberty in DYT6 dystonia. Considering the spread of manifestation ages up into adulthood, non-manifesting subjects were required to be 30 years at minimum.

B. RESULTS

B.1. Regional Metabolism in Primary Torsion Dystonia: Effects of penetrance and Genotype. (Carbon et al. 2004a)

To reduce the heterogeneity of findings of various dystonia cohorts, genotype specific abnormalities were sought in specified subgroups. Eidelberg and colleagues (1995) identified a network of symptom-specific metabolic increases in the lateral frontal and paracentral cortices, and the striatum, pons and midbrain in idiopathic, predominantly right-sided dystonia. It was however unclear, whether or how many DYT1 positive subjects had been included in that study, as this genotype had not been identified at the time of the study. After the identification of the DYT1 genotype as one genetic variant of idiopathic generalized dystonia (Ozelius et al., 1997), a first DYT1-specific metabolic brain network was described using volume-of-interest measurements in conjunction with principal components analysis (Eidelberg et al. 1998). This network was characterized by relative metabolic increases in the posterior putamen, globus pallidus, cerebellum and SMA in patients with focal or generalized DYT1 dystonia (Eidelberg et al., 1998) and subsequently validated on a voxel-based level (Trost et al., 2002).

In the amended study, DYT1-specific metabolic changes were compared to the metabolic topography in the DYT6 genotype. Based on preliminary studies in this genotype (Trost et al. 2002), and based on the clinical similarities of both genotypes, we hypothesized that metabolic abnormalities would largely overlap. ^{18}F-FDG PET was used to scan 12 MAN-DYT1 and 11 NM-DYT1, six NM-DYT6 and seven MAN-DYT6, as well as 11 control subjects. The data from all five groups were analyzed with SPM99 and analysis of variance (ANOVA) with posthoc contrasts.

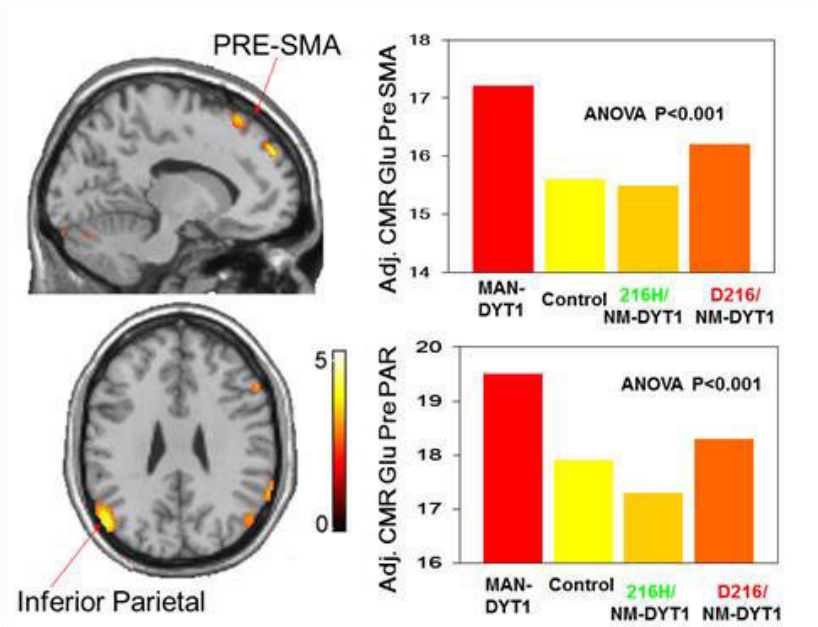
Contrasting to our hypothesis, we found a dissociation of metabolic changes related to phenotype and genotype. Manifesting gene carriers of both genotypes exhibited bilateral hypermetabolism in the presupplementary motor area (BA 6) and parietal association cortices (BA 40/7) compared with the respective non-manifesting counterparts. By contrast, genotype-specific increases in DYT1 mutation carriers (manifesting and non-manifesting combined, with no within-group differences) were localized to the putamen, anterior cingulate (BA 24/32), and to both cerebellar hemispheres. The DYT6 genotype showed opposing metabolic changes, involving hypometabolism of the putamen and hypermetabolism in the temporal cortex (BA 21).

As a conclusion, we suggested that the susceptibility to dystonia related to an imbalance of cerebellar and basal ganglia metabolism. Moreover, symptoms of dystonia may be

associated with abnormal movement preparation reflected by metabolic increases in the motor preparatory cortices and relating to defective sensorimotor integration reflected by metabolic increases in parietal association cortices.

Well after this publication, sequence variants within the coding region of the DYT1 gene were identified (Kock et al., 2006). One of these variants, a single nucleotide polymorphism, occurring in 12% of subjects, consisted in a substitution of aspartic acid (D) for histidine (H216, Kock et al., 2006). The H216 polymorphism was found to reduce cell inclusions that are typically seen in cells expressing the DYT1 mutation (ΔGAG). Interestingly, Risch and colleagues (2007) found an unexpected proportion of NM-DYT1 to carry this additional polymorphism. Thus, the H216 polymorphism was suggested to be protective with regard to dystonia pathology. In a pilot study, we tested the effect of this protective polymorphism in our cohort of NM-DYT1 (Carbon et al., 2008c). Resting state FDG PET scans were performed in 13 MAN-DYT1 (age 39.9 ± 15.1 years), three 216H/NM-DYT1, five D216/NM-DYT1; NM-DYT1 age 55.9 ± 14.7 years), and 12 healthy controls matched to NM-DYT1 (age 52.7 ± 13.8 years). Volume of interest (VOI) based measurements of the phenotype specific regions, identified earlier (Carbon et al., 2004a) were performed. We found metabolic values of pre-SMA and inferior parietal cortex (i.e. phenotype specific regions) in unprotected mutation carriers (216D-NM-DYT1) to fall within the range of MAN-DYT1. By contrast, those carrying the protective polymorphism (H216-NM-DYT1) showed metabolic values within the control range in the respective regions (see Figure 1; Carbon et al. 2008c).

Figure 1: Comparison of glucose metabolism in phenotype specific volumes of interest from ^{18}F-FDG PET data in NM-DYT1 with the 216H compared to the D216 polymorphism.



p. 18 - 24:

Carbon M, Su S, Dhawan V, Raymond D, Bressman S, Eidelberg D. Regional metabolism in primary torsion dystonia: effects of penetrance and genotype. Neurology. 2004 Apr 27;62(8):1384-90. PubMed PMID: 15111678. DOI 10.1212/01.WNL.0000120541.97467.FE

B.2. Metabolic Changes in DYT11 Myoclonus-Dystonia. (Carbon et al., 2013)

Expanding on the findings in the DYT1 and the DYT6 genotype, we aimed to identify brain regions with metabolic abnormalities in a dystonia plus syndrome, i.e. in DYT11 myoclonus-dystonia (DYT11-MD). We also compared these metabolic abnormalities in DYT11-MD with those found in other forms of hereditary dystonia and in posthypoxic myoclonus.

As in our earlier study, ^{18}F-FDG PET of the brain during the resting state was performed in six subjects with DYT11-MD (age 30.5 ± 10.1 years) and in 6 NM DYT11 (age 59.1 ± 8.9 years) representing the parental generation of the affected individuals. We used voxel-based whole brain searches implemented in SPM5 to compare these data to scan data from age-matched healthy control subjects. As a secondary analysis, we searched for shared characteristics of either one of the hereditary dystonias and the DYT11 dystonia-plus syndrome; or shared characteristics of posthypoxic dystonia and the DYT11 dystonia-plus syndrome. These overlapping abnormalities were identified by comparisons of the DYT11-MD subjects to hereditary dystonias (DYT1, DYT6, DRD) and to posthypoxic myoclonus.

Surprisingly, we found DYT11 genotype-specific metabolic abnormalities. This finding was completely unexpected as NM-DYT11 are expected not to show any changes in epsilon-sarcoglycan, since gene expression is thought to be strictly regulated via maternal imprint. The genotype-specific metabolic abnormalities, however, were present independently in comparisons of the paternal as well as the filial (manifesting) generation with age-matched healthy subjects respectively. The significant DYT11 genotype-specific changes included metabolic increases in the inferior pons and in the posterior thalamus as well as reductions in the ventromedial PFC. As with the DYT1 and the DYT6 genotype, additional significant phenotype-related increases were present. These were localized to the parasagittal cerebellum. Interestingly, this latter abnormality was shared only with posthypoxic myoclonus, but not with other forms of hereditary dystonia. By contrast, all dystonia cohorts exhibited significant metabolic increases in the superior parietal lobule in concordance with the metabolic abnormalities localized to this region in the earlier study of DYT1 and DYT6 dystonia.

The manifestation related findings were highly consistent with earlier imaging reports on single cases with DYT11 dystonia (Nitschke et al., 2006; Tai et al., 2009). They were consistent with a subcortical myoclonus generator in DYT11-MD, likely involving the cerebellum. Such a generator had been suggested in a preceding neurophysiological study (Hubsch et al., 2011).

Contrasting to the genotype specific findings, rather subtle increases in the superior parietal cortex related to the additional presence of dystonic symptoms, again most likely pointing to a deficit of sensorimotor integration in dystonia.

Despite clear evidence for the regulation of penetrance in DYT11-MD with maternal imprinting of the epsilon-sarcoglycan gene (Müller et al., 2002), NM-DYT11 (who should thus not express the mutated protein) showed significant metabolic abnormalities that were not explained by this genetic model. Indeed, these findings were very difficult to reconcile with this notion of maternal imprinting of the SGCE gene. It has to be noted though, that abnormal motor activation responses obtained during f-MRI had been reported in NM-DYT11 earlier (Beukers et al., 2010, 2011), similarly questioning the dogma of maternal imprint as the sole regulator of penetrance during adulthood. In our discussion on this topic we suggested, that maternal imprinting is not a binary, all-or-nothing regulator of gene penetrance. To support this idea, evidence for variations of maternal imprinting by species, tissue, and developmental stage is detailed in the manuscript. Consistent with the idea of hereditary dystonias as neurodevelopmental disorders (disease onset of DYT11 dystonia is typically during childhood or adolescence), imprinted genes have been shown to play an important role during embryogenesis and neurodevelopment. This means that the expression of imprinted genes can change during development, tissue differentiation and during disease (Taniguchi et al., 1995; Davies et al., 2005; Schulz et al., 2006; Hudson et al., 2010). In particular, a loss of imprinted expression during cellular differentiation can be present in a tissue-specific manner. For epsilon-sarcoglycan, a weak (non-expected) maternal expression, in addition to the (expected) paternal expression, has been shown in adult rodent neuronal cells (Piras et al., 2000), even though the maternal imprint was maintained in neonatal and embryonic brain tissue. We have thus suggested from our data, that it is possible that a biallelic expression of epsilon-sarcoglycan is needed during certain periods of neurodevelopment, during which a loss-of-function mutation in the maternal allele (as is posited for DYT11-MD) causes the metabolic abnormalities recorded in our study.

p. 27 - 33.

Carbon M, Raymond D, Ozelius L, Saunders-Pullman R, Frucht S, Dhawan V, Bressman S, idelberg D. Metabolic changes in DYT11 myoclonus-dystonia. Neurology. 2013 Jan 22;80(4):385-91. doi: 10.1212/WNL.0b013e31827f0798. Epub 2013 Jan 2. PubMed PMID: 23284065; PubMed Central PMCID: PMC3589244.

B.3. Microstructural White Matter Changes in Carriers of the DYT1 Mutation.

(Carbon et al. 2004b)

This study was the very first DTI study in dystonia. The aim of this study was to test the hypothesis that the DYT1 genotype was associated with a disorder of mirco-anatomical connectivity as a consequence of a neurodevelopmental disruption. At the time of the study this hypothesis was based (1) on the clinical observation of the typical age of onset in DYT1 dystonia during childhood or adolescence, a time of intense synaptic pruning (Bressman 2004), (2) on the early notion of a role for the nematode homologue of torsinA in cytoskeletal dynamics (Basham and Rose 2001; Ferrari Toninelli et al. 2003) and (3) on the lack of functional network expression during learning in asymptomatic mutation carriers (Carbon et al. 2002; for details see B.10., B.11.).

We used DTI on a 1.5 Tesla MRI scanner to assess measures of diffusivity as the basis of diffusion tensor calculation in white matter pathways in 12 DYT1 mutation carriers (age: 44.1 ± 17.8 years) and in 17 age-matched control subjects (age: 43.3 ± 17.0 years). Out of the twelve DYT1 mutation carriers, eight were non-manifesting (age: 53.4 ± 13.9 years) and four were manifesting (age: 25.5 ± 17.8 years). Voxel-based analyses showed FA reductions ($p < 0.005$) in the subgyral white matter of the SMC of DYT1 carriers. Due to the small number of manifesting carriers, no analyses regarding MAN-DYT1 vs. NM-DYT1 were performed.

These results were the first to suggest micro-structural in vivo correlates of genotype specific changes. They also contributed to the discussion of the role of torsinA in neurodevelopment. Interestingly, concomitant to the publication of the results in humans, the molecular role of torsinA was found to relate closely to the nuclear envelope, which in turn has a crucial role in neurodevelopment (Gerace, 2004; Goodchild and Dauer 2004). Subsequently, a line of research has confirmed and expanded the suggested role of torsinA in neurodevelopment. Nery and colleagues (2008) found that mutant torsinA, unlike wild-type torsinA, clustered very strongly with nesprin-3 (a molecule spanning the outer nuclear membrane). They also assessed the orientation of the centrosome relative to the nucleus and the leading edge of dividing cells. In wild type torsinA containing cells the vast majority was correctly polarized. By contrast, mutant torsinA containing cells showed the correct polarization only in around 40% (Nery et al. 2008).

Regarding the work in humans, this study was the basis for a series of subsequent manuscripts, exploring microstructural correlates of symptom manifestation in DYT1 dystonia (Carbon et al. 2008, Argyelan/Carbon et al. 2009, Vo et al. submitted to J Nsci 2013) and in other dystonias (see also C.1.b.).

p. 35 – 38:

Carbon M, Kingsley PB, Su S, Smith GS, Spetsieris P, Bressman S, Eidelberg D. Microstructural white matter changes in carriers of the DYT1 gene mutation. Ann Neurol. 2004 Aug;56(2):283-6. PubMed PMID: 15293281. DOI: 10.1002/ana.20177

B.4. Microstructural White Matter Changes in Primary Torsion Dystonia. (Carbon et al., 2008)

Based on the observation of reduced microstructural connectivity in the subgyral white matter of the SMC (Carbon et al. 2004), we aimed to assess whether additional microstructural changes were present within motor control pathways in subjects manifesting with dystonia.
Thus, in this study subjects carrying the DYT1 or the DYT6 mutation were included. Specifically, seven subjects with segmental or generalized dystonia (age: 29.3 ± 9.8 years; four DYT1 and three DYT6 mutation carriers) were compared to seven age-matched controls (30.2 ± 5.8 years). We used an established DTI protocol at 1.5 Tesla (Lim et al., 1999) and voxel-based analyses to assess the microstructure of white matter.
Measures of FA were significantly reduced in the dystonia patients in the pontine brainstem in the vicinity of the left superior cerebellar peduncle and bilaterally in the white matter of the sensorimotor region. It has to be noted, that the spatial resolution of MRI and as a consequence the projection of statistical maps, as the source of localization of imaging findings is of limited quality in the brainstem. Nevertheless, these findings might be related either to cerebellar outflow pathways as detailed later (Argyelan/Carbon et al. 2009) or to the presence of ubiquitin positive inclusions in the vicinity of the pedunculopontine nucleus (PPN) that had been shown in human manifesting DYT1 carriers (Mc Naught et al., 2004) and in a transgenic mouse model of DYT1 dystonia (Shasidaran et al., 2005). The interpretation of these results is complicated as the PPN cannot be regarded a homogenous structure. It has irregular boundaries and it has particularly long ranging axons, projecting to removed targets, such as the spinal cord or forebrain structures, but it is also closely interrelated with the basal ganglia (Pahapill and Lozano 2000; Martinez-Gonzalez et al., 2011). Ultimately, the observed reductions in brainstem FA were related to the superior cerebellar peduncle, which is the main cerebellar outflow structure (Argyelan/Carbon 2009; B.5.).

p 40 – 45:

Carbon M, Kingsley PB, Tang C, Bressman S, Eidelberg D. Microstructural white matter changes in primary torsion dystonia. Mov Disord. 2008 Jan 30;23(2):234-9. PubMed PMID: 17999428. DOI 10.1002/mds.21806

B.5. Cerebellothalamocortical Connectivity Regulates Penetrance in Dystonia.

(Argyelan M*, Carbon M*, et al., 2009. *equal author contribution)

The finding of reduced brainstem connectivity in DYT1 and DYT6 dystonia needed to be attributed to a pathway, to understand its functional role within the pathophysiology of dystonia. The DTI studies on the 1.5 Tesla MRI scanner were limited in this regard; a 5 mm skip had been used between each plane during image acquisition to cover the brain volume from vertex to brainstem. Thus vector connections could not be calculated.

The data for this subsequent study were therefore collected at another MRI scanner at 3 Tesla, to enable the use of probabilistic tractography (Behrens et al., 2007). For this study manifesting subjects (n = 12: 7 DYT1/5 DYT6; age 42.2 ± 15.2) as well as non-manifesting subjects (n = 8: 4 DYT1/4 DYT6; age 44.0 ± 17.4) were recruited. Seed regions for probabilistic tractography were defined based on our earlier DTI studies in the cerebellar outflow pathway. Due to the technical difficulty to calculate vector trajectories past a decussation (Johansen-Berg and Behrens, 2006), two seed masks (for each cerebellar outflow side) were created for each subject; one below and one above the decussation of the cerebellar peduncles. From these seed masks, four individual probabilistic tracts were constructed for each participant. Voxel-based group comparisons of connectivity measures of these tracts revealed reduced integrity of cerebello-thalamo-cortical fiber tracts, in both manifesting and clinically non-manifesting dystonia mutation carriers in the very proximal segment of the superior cerebellar peduncle (Fig. 2 of the amended manuscript). Confirming our earlier observations, these reductions were more pronounced in manifesting than in non-manifesting mutation carriers.

Non-manifesting mutation carriers were additionally distinguished by an area of fiber tract disruption situated distally along the thalamo-cortical segment of the pathway, in tandem with the proximal cerebellar outflow abnormality. Modeling of clinical penetrance showed that in individual gene carriers, clinical penetrance was determined by the difference in connectivity measured at these two sites. In secondary analyses, the functional role of these reductions was explored using correlation analyses of the connectivity measures and brain activation responses recorded during standardized motor tasks with $H_2{}^{15}O$ PET (as detailed in chapter B.7). Connectivity measures from a cluster close to the dentate nucleus (see cluster a1 in figure 1 of the amended manuscript) correlated with increased motor activation in the cerebellar nuclei, the thalamus and SMC, consistent with a loss of inhibition at the cortical level.

In summary, the DTI-MRI studies support the idea of hereditary dystonias as neurodevelopmental circuit disorders, rather than the idea of isolated basal ganglia pathology.

p 47 – 54:

*Carbon M; *Argyelan M, Niethammer M, Ulug AM, Voss HU, Bressman SB, Dhawan V, Eidelberg D. Cerebellothalamocortical connectivity regulates penetrance in dystonia. J Neurosci. 2009 Aug 5;29(31):9740-7. doi: 10.1523/JNEUROSCI.2300-09.2009. PubMed PMID: 19657027; PubMed Central PMCID: PMC2745646.

B.6. Abnormal Striatal and Thalamic Dopamine Neurotransmission: Genotype-Related Features of Dystonia. (Carbon et al., 2009)

As highlighted in the general introduction, there is ample evidence to support the idea of a major role of dopaminergic transmission in the dystonias (Burke et al., 1982; Factor and Matthews, 1991; Hierholzer et al.; 1994; Ernst et al., 1996; Wong et al., 1996; Naumann et al.; 1998; Brashear et al., 1999; Casey, 2004; Perlmutter et al., 1997; Kühn et al., 2008). Indeed, a disturbance of the dopaminergic system is the only etiology present in DRD, or in tardive dystonias subsequent to D_2 receptor blocking agent exposure. Nevertheless, earlier studies in primary dystonias had only shown minor changes within the dopaminergic system. In particular, an earlier study in NM-DYT1 (Asanuma et al., 2005), showed a 15% reduction of D_2 receptor binding in caudate and putamen. The present study was performed to assess whether changes in D_2 receptor availability would determine symptom manifestation in carriers of mutations for primary dystonia.

Manifesting and non-manifesting carriers of the DYT1 and DYT6 genotype were scanned with [(11)C] raclopride (RAC) and PET during rest. Measures of D_2 receptor availability in the caudate nucleus and putamen were determined using a standardized region-of-interest approach with explicit age correction (Asanuma et al. 2005). Values from mutation carriers and healthy controls were compared using analysis of variance to assess the effects of genotype and phenotype. Additionally, voxel-based whole brain searches were conducted to detect group differences in extrastriatal regions.

Our data showed significant reductions of caudate and putamen D_2 receptor availability in both genotypes independently compared to healthy controls ($p< 0.001$). The changes were more pronounced in DYT6 than in DYT1 carriers (-38.0 +/-3.0% vs -15.0 +/- 3.0%, $p < 0.001$). Importantly, dopaminergic changes did not relate to symptom manifestation, as there were no differences between manifesting and non-manifesting carriers of either genotype.

Voxel-based analysis additionally revealed reduced RAC binding in the ventrolateral thalamus of both groups of mutation carriers. As in the striatum, the thalamic binding reductions were more pronounced in DYT6 carriers and were not influenced by the presence of clinical manifestations.

In human hereditary dystonia, it is possible that the reduced D_2 receptor availability results from (1) dysfunction of D_2-bearing neurons, (2) or loss of D_2-bearing neurons, (3) increased synaptic dopamine levels, or (4) a combination of the mechanisms. These changes, which may be present to different degrees in the DYT1 and DYT6 genotypes, are likely to represent only one aspect of the susceptibility factors in mutation carriers. Consistent with limited clinical efficacy of dopamine depleting agents in these genotypes, a salient role of the dopaminergic system in disease control can likely be dismissed.

p 56 – 62:

Carbon M, Niethammer M, Peng S, Raymond D, Dhawan V, Chaly T, Ma Y, Bressman S, Eidelberg D. Abnormal striatal and thalamic dopamine neurotransmission: Genotype-related features of dystonia. Neurology. 2009 Jun 16;72(24):2097-103. doi: 10.1212/WNL.0b013e3181aa538f. PubMed PMID: 19528516; PubMed Central PMCID: PMC2697963.

B.7. Increased Sensorimotor Network Activity in DYT1 Dystonia: a Functional Imaging Study. (Carbon et al., 2010)

Neurophysiological studies have provided evidence of a loss of inhibition at the level of the primary motor cortex in primary dystonia, but data from functional imaging studies in primary dystonia are highly contradictory (see chapter A.2.c./d.).

To address this problem on a systems level, we measured sensorimotor activation during a paced, kinematically controlled motor task (Ghilardi et al., 2000) with $H_2{}^{15}O$ PET in DYT1 mutation carriers (11 MAN-DYT1 predominantly with generalized dystonia, age: 42.8 ± 15.5 years and 10 NM-DYT1, age: 51.5 ± 14.3 years) and in age-matched controls (n=12; age: 44.7 ± 12.7 years). Imaging data and behavioral data were obtained and analyzed in this study.

The motor task consisted in reaching regularly for eight circularly arrayed targets on a digitizing tablet in a counter clockwise manner (target array as detailed in Figure 4; **B.8**). This task can be performed even in the presence of dystonia and movement kinematics are recorded during the task to enable movement analyses and correlation of motor characteristics with brain activation measures. Importantly, the control condition did not consist of complete resting, but was matched with regard to the audio-visual input to the motor task. This had been designed to clearly isolate motor related activation as opposed the sensory input related to the standardized task. Within- and between-group contrasts were analyzed with SPM 5. For network analysis, we used ordinal trends analysis (Habeck et al., 2005) and first identified a normal motor-related activation pattern (NMRP) in a set of 39 motor and audio-visual scans acquired in an independent cohort of 18 healthy volunteer subjects. Ordinal trends analysis is a supervised principal components analysis technique designed to identify spatial covariance patterns that change in expression within individual subjects across experimental conditions. The expression of this pattern was then prospectively quantified in the motor and control scans acquired in each of the gene carriers and controls, using automated prospective network calculation. Network values for controls, NM-DYT1 and MAN-DYT1 were compared with ANOVA and post hoc contrasts.

Voxel-wise comparison of DYT1 carriers and controls revealed abnormally increased motor activation responses ($P < 0.05$, corrected in SPM), in the SMC, dorsal PreMC, SMA and the inferior parietal cortex. Network analysis of the normative derivation cohort identified the normal motor-related activation pattern topography (i.e the covariant network activity that showed a significant increase from rest to motor activation in all subject, $P < 0.0001$; NMRP). This NMRP was characterized by covarying neural activity in the SMC, dorsal PreMC, SMA and cerebellum (Figure 2 in the amended manuscript). In the DYT1 study cohort, prospective

network calculation showed that NMRP expression during movement was abnormally elevated in the MAN-DYT1 ($P < 0.001$) but not in NM-DYT1 (Figure 3 in the amended manuscript). In contrast, in the non-motor control condition, abnormal increases in NMRP network activity were present in both groups of gene carriers ($P < 0.001$). In this condition, NMRP expression in non-manifesting carriers was greater than in controls, but lower than in affected carriers (Figure 3 in the amended manuscript). Importantly, NMRP expression in the audio-visual condition correlated with independent dystonia clinical ratings ($r = 0.70$, $P = 0.04$).

In addition to the image analysis, kinematic data were analyzed: As expected, general motor task performance, expressed as the percentage of correctly hit targets, was reduced in MAN-DYT1 (49.4 ±11.4%, mean ± SD) compared to controls as well as to their non-manifesting counterparts (controls: 85.1 ± 7.2%; non-manifesting DYT1: 77.4 ± 4.8; $p=0.004$). Moreover, dystonics showed a greater movement irregularity of the repetitive reaching movements, expressed as a higher individual standard deviation of movement time (defined as the time from movement onset to the end point for an individual reaching movement to one target; movement time_SD: MAN-DYT1: 103.6 ± 9.0ms; NM-DYT1: 69.7±11.3; controls: 60.5 ± 12.0ms; $P = 0.003$). All other motor kinematics did not differ significantly from controls. Importantly, no abnormalities of motor kinematics were recorded in the NM-DYT1 group.

As mentioned above (Chapter B.5), there was a close correlation of functional motor abnormalities (i.e. SMC and PreMC activation) and microstructural abnormalities within the cerebellar outflow pathway. This association was also confirmed in the reverse approach: In an auxillary analysis, we used measures of NMRP during the AV condition as covariates for FA maps in nine DYT1 mutation carriers. Whole brain voxel-based analyses disclosed a significant negative correlation in the dorsal brainstem in vicinity of cerebellar outflow pathways ($R^2= 0.82$ $p=0.006$).

Conversely, we also studied associations of measures of D_2 receptor availability obtained in earlier studies (cf. chapter B.6), however, neither NMRP expression, nor behavioral motor kinematic characteristics were correlated with either measures of caudate or putamen D2 receptor availability ($R<0.2$; $p>0.2$ for all analyses).

p. 65 – 75:

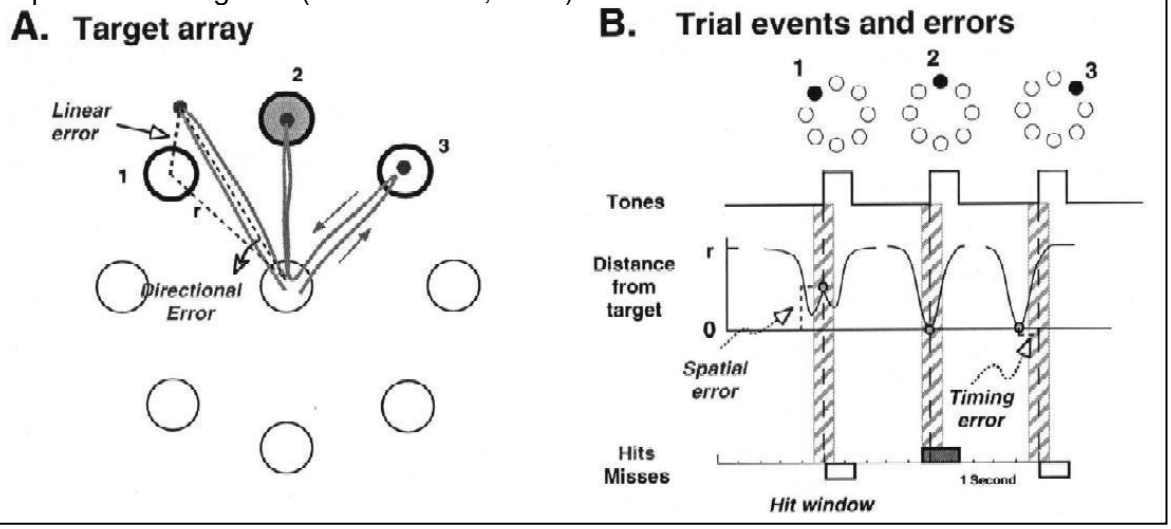
Carbon M, Argyelan M, Habeck C, Ghilardi MF, Fitzpatrick T, Dhawan V, Pourfar M, Bressman SB, Eidelberg D. Increased sensorimotor network activity in DYT1 dystonia: a functional imaging study. Brain. 2010 Mar;133(Pt 3):690-700. doi: 10.1093/brain/awq017. Epub 2010 Mar 5. PubMed PMID: 20207699; PubMed Central PMCID: PMC2842516.

B.8. Increased Cerebellar Activation during Sequence Learning in DYT1 Carriers: an Equiperformance Study. (Carbon et al., 2008b).

Sequence learning experiments have long been established as probes for basal ganglia function (for review wee Hikosaka et al., 1999; Hardwick et al., 2013), and have thus been of interest for studies in movement disorders.

For the purpose of this study sequence learning-related network activation was first established in health (Ghilardi et al., 2000; Carbon et al. 2003) and the modulation of this network activity was tested in Parkinson's disease ON and OFF treatment (Nakamura et al., 2001 Carbon et al. 2003).

Figure 2: Target array and movement recording for the motor reaching and the motor sequence learning task (Ghilardi et al., 2000)



In these tasks (detailed in Ghilardi et al., 2000) the hand path for the reaching movements was recorded on a digitizing tablet. For the simple motor task (see figure 4) subjects had to reach for the serially highlighting targets at a pace of 1/s (enforced by a tone sounding in the same rhythm). The targets were highlighted in counterclockwise order in a predictable manner, so subjects could plan the movement and perform the reaching in a rhythmical way to arrive at the target in the correct time frame (signaled to the subject by a change in color of the target). By contrast, for the motor sequence learning task (MSEQ; using the same target array, see figure 4) eight targets per cycle were highlighted in an unpredictable, but repetitive manner. The subjects had to try to reach for the target within the correct time frame (1/ s as in the simple motor task), making this task a reaction time task for the first cycle of the target appearances. During the repeating cycles though, subjects achieved explicit and implicit knowledge of target positions and sequences could thus progressively be learned. Subjects were instructed that a repeating pattern of eight targets was to be learnt. Any target that was reached in an anticipatory manner (i.e. movement onset was recorded before target appearance) counted as a learnt target. The sum of all correctly anticipated movements

during one trial block was termed the global retrieval index. An additional learning index, the acquisition index was also defined: Any correctly hit target that had not been successfully anticipated in the previous cycled counted an acquired target.

Having established the normal acquisition and retrieval networks in health and in PD (Carbon et al. 2003), we expected to find a similar modification of networks in hereditary dystonia, since this was considered mostly a basal ganglia disorder. However, neither the acquisition network, nor the retrieval network was expressed in DYT1 mutation carriers (Carbon et al., 2004d, 2007). Instead, increased cerebellar recruitment was recorded, both at the network level (Carbon et al. 2007) as well as measured in voxel-based group comparisons (Ghilardi et al., 2003; Carbon et al., 2008, 2011).
Group comparisons during sequence in NM-DYT1 vs. controls found that despite comparable motor performance, sequence learning in MSEQ was reduced in NM-DYT1 relative to age-matched controls (Ghilardi et al., 2003). During sequence learning, activation responses in DYT1 carriers were increased in the left ventral PFC cortex, and lateral cerebellum. These findings suggested that abnormalities in sensorimotor integration and brain function exist in NM-DYT1, and that these manifest as defect in motor sequence learning.
In addition to the univariate group comparisons (Ghilardi et al., 2003), network analyses (as detailed above) were used in this dataset to test for normal network expression in DYT1 mutation carriers (Carbon et al., 2002; Carbon et al., 2004d). Surprisingly, and in stark contrast to PD (as detailed in chapter B.8), network-performance relationships were absent in NM-DYT1. Instead a DYT1 specific network was identified, characterized by covarying motor sequence learning specific activation in the SMC, DLPFC, anterior cerebellar cortex and cerebellar dentate nucleus. Contrasting to healthy controls there was no contribution of the basal ganglia in the DYT1 specific network, and there was a stronger contribution of the cerebellum (Carbon et al., 2002). As this cerebellar shift was absent in the neurodegenerative disorder, we hypothesized that an early neurodevelopmental process might relate to cerebellar shift during learning in the DYT1 genotype.

In the subsequent study (Carbon et al., 2008b) we used a modified version of the earlier sequence learning task MSEQ. In this task the targets appearing in the same target array as detailed above were not presented sequentially, but were to be discovered by trial-and-error. We chose to alter the task, because trial and error learning depends strongly on cerebellar input (Doya 2000). Moreover, this trial-and-error sequence-learning task (TSEQ) could be adapted in difficulty to match the mutation carrier group and controls to the same level of performance (i.e. controls perform subjectively easy tasks, which can then be matched to the

performance level of the disease group). An equiperformance study design (c.f. Mentis et al., 2003) is suited to identify neural resources that are used to achieve performance within the normal range. Six NM-DYT1 and six control subjects were scanned with $H_2{}^{15}O$ PET during TSEQ and a matched motor execution task. PET data analysis was performed using SPM99.

Although performing at matched levels, NM-DYT1 overactivated the lateral cerebellum and the right inferotemporal cortex relative to age-matched controls. In contrast, they showed relative activation deficits in DLPFC bilaterally, as well as in the left anterior cingulate and the dorsal premotor cortex.

The prominent compensatory involvement of the cerebellum during target learning confirmed our earlier results in NM-DYT1 (Ghilardi et al., 2003; Carbon et al., 2004d.). Contrasting to mutation carriers, normals used bilateral cerebellar activation in conjunction with a prominent prefrontal bilateralization when confronted with a much higher task difficulty. That means, that this compensatory cerebellar activation could be interpreted as a normal sign of adaptive cerebral plasticity. However, as (1) dystonia has been related to abnormally increased plasticity on many levels (Quartarone et al. 2006) and (2) dystonia has been linked to cerebellar pathology (LeDoux 2003), we suggested that this cerebellar overactivation reflects a maladaptive neuroplastic response.

p. 79 -87:

Carbon M, Ghilardi MF, Argyelan M, Dhawan V, Bressman SB, Eidelberg D. Increased cerebellar activation during sequence learning in DYT1 carriers: an equiperformance study. Brain. 2008 Jan;131(Pt 1):146-54. Epub 2007 Oct 18. PubMed PMID: 17947338.
doi: 10.1093/brain/awm243

B.9. Impaired Sequence Learning in Dystonia Mutation Carriers: a Genotypic Effect. (Carbon et al., 2011)

We expanded the observations in NM-DYT1 to MAN DYT1 to explore the effect of genotype and phenotype in sequence learning abnormalities. In the current study, we assessed whether sequence learning deficits were also present in clinically manifesting DYT1 carriers or carriers of other primary dystonia mutations such as the DYT6 genotype. Additionally, we determined whether sequence learning performance and associated brain activation in these subjects correlate with previously described genotype-related abnormalities of cerebellar pathway integrity (as detailed in chapter B. 5) and striatal D2 dopamine receptor binding (as detailed in chapter B.6). Nineteen DYT1 carriers (10 NM DYT1; age: 51.5±15.1 years; nine MAN DYT1; age: 46.1±15.1 years) and 12 healthy control subjects (age: 42.8 ±15.3 years) were scanned with $H_2{}^{15}O$ PET while performing the motor sequence learning task MSEQ and an observational sequence learning task (VSEQ) as well as respectively matched reference tasks (as detailed in chapter B.8). Eleven DYT6 carriers (four NM-DYT6: age 38.0 ± 22.1 years; seven manifesting DYT6: 35.3±14.2 years) were evaluated during task performance without concurrent PET imaging. Subgroups of DYT1 and DYT6 carriers also underwent DTI MRI and ^{11}C-raclopride PET to measure caudate/putamen D_2 receptor binding. These imaging measures were correlated with sequence learning performance and associated activation responses.

There was no effect of symptom manifestation on sequence learning performance. Sequence learning deficits of similar magnitude were observed in MAN-DYT1 and NM-DYT1. Importantly, however, despite major similarities in microstructural abnormalities, learning deficits were not detected in DYT6 carriers, irrespective of clinical penetrance. Voxel-based image analyses (SPM 5) showed significant increases in sequence learning-related activation in the left lateral cerebellar cortex and in the right premotor and inferior parietal regions in MAN-DYT1 relative to controls. Increases in premotor cortical activation observed in the mutation carriers correlated with reductions in cerebellar pathway integrity measured using magnetic resonance diffusion tensor imaging and probabilistic tractography. Additionally, the cerebellar tract changes correlated with reductions in dentate nucleus activation recorded during task performance. By contrast, sequence learning performance and task-related activation responses did not correlate with striatal D_2 receptor binding, even though a close relationship between these measures had been shown in an earlier study in controls and in early unmedicated Parkinson's disease patients (Carbon et al. 2004c).

The role of the cerebellum in modulating neocortical activation is a current topic of research, in particular, the mechanism by which cerebellum and cortex interact during learning in health, warrant further investigation (Koziol et al. 2013; Bostan et al. 2013). A deeper

understanding of these mechanisms would enable a more informed interpretation of our findings in DYT1 dystonia.

In summary, we found that sequence learning deficits and concomitant increases in cerebellar activation are specific features of the DYT1 genotype. The close relationship between reduced cerebellar pathway integrity and increased learning-related activation of the premotor cortex is compatible with the view of DYT1 dystonia as a neurodevelopmental circuit disorder.

p. 90 – 101:

Carbon M, Argyelan M, Ghilardi MF, Mattis P, Dhawan V, Bressman S, Eidelberg D. Impaired sequence learning in dystonia mutation carriers: a genotypic effect. Brain. 2011 May;134 (Pt 5):1416-27. doi: 10.1093/brain/awr060. Epub 2011 Apr 22. PubMed PMID: 21515903; PubMed Central PMCID: PMC3097890.

C. DISCUSSION

Using a multi-modal imaging approach in hereditary dystonias, this series of studies supported the overarching hypotheses, showing that metabolic, microstructural, functional changes reach well beyond an isolated affection of the indirect striatal pathway, as suggested for hyperkinetic disorders traditionally (Albin et al., 1989). Similarly, a predominant role of dopaminergic neurotransmission within the basal ganglia was dismissed as hypothesized.

We demonstrated that functional changes of the striatum, both at the metabolic and the neurochemical level reflect the susceptibility for primary torsion dystonia (Carbon et al., 2004a, Carbon et al., 2009, Carbon et al., 2011). By contrast, penetrance and disease severity did not relate to striatal characteristics, but were rather associated with the expression of a cortical sensorimotor brain activation network (Carbon et al., 2011). Importantly, microstructural abnormalities underlying the expression of the cortical sensorimotor network were found in the cerebellar outflow pathways in dystonia mutation carriers (Carbon et al. 2008a; Argyelan/Carbon et al., 2009). Interestingly, we found a novel interaction of cerebello-thalamic and thalamo-cortical connectivity as a potential mechanism regulating penetrance in the DYT1 and DYT6 genotype (Argyelan/Carbon et al., 2009). Thus, our data suggest that the functional entity of sensorimotor circuitry rather than one node within this network regulates symptom expression in dystonia.

C.1. Metabolic and Microstructural Changes

The results of our studies have shed additional light on the disturbance of neural circuitry and the abnormal structure/function relationships that characterize dystonia. Localization of circuit changes adds to the discussion on new therapeutic targets and treatment strategies for this disorder. Unfortunately, rather than supporting the idea of a prevailing, unifying pathology for the hereditary dystonias, the FDG-studies (Carbon et al., 2004a, 2013) have demonstrated striking disparities of brain glucose metabolism between hereditary dystonias. The DYT1 genotype is characterized by striatal and cerebellar metabolic increases, whereas metabolic reductions in the same areas are found in the DYT6 genotype. Moreover, conjunction analyses across all genotypes assessed (Carbon et al., 2013) identified only a small region within the parietal association cortices as a shared metabolic feature of dystonia.

In summary, three major elements emerge from the line of work on metabolism and microstructure in hereditary dystonias: (1) cerebellar abnormalities (in addition to striatal

changes), (2) microstructural abnormalities within cerebello-thalamo-cortical circuits (3) parietal cortex abnormalities.

C.1.a. Cerebellar Abnormalities

While abnormal inferior cerebellar cortex hypermetabolism primarily characterized susceptibility in DYT1 dystonia (Carbon et al., 2004a), network analyses showed an interaction of trait and state features with relative cerebellar hypometabolism within the DYT1 and DYT6 genotype (Carbon and Eidelberg, 2006, 2009).

By contrast, within the DYT11 genotype, parasagittal cerebellar hypermetabolism was found to be related to symptom expression. Importantly, however, this region was likely more related to the presence of myoclonus, as it was a shared characteristic with posthypoxic myoclonus and the shared abnormality with dystonia was localized to the parietal cortex (Carbon et al., 2013).

The results from DYT1 positive humans have since been tested in a transgenic DYT1 knock-in mouse model (Ulug et al., 2011). Heterozygous knock-in mice and littermate controls underwent microPET followed by ex vivo high-field DTI and tractographic analysis. As detailed in the introduction (A.2.b) these mutant mice fail to develop clinical correlates of dystonia or other abnormal movements. Nevertheless, the DYT1 knock-in mice showed significant cerebello-thalamo-cortical tract changes and abnormalities in brainstem regions linking cerebellar and basal ganglia motor circuits, clearly similar to the abnormalities identified earlier in humans (Carbon et al., 2008a, Argyelan et al., 2009). In addition, as in humans, measures of SMC activity (metabolic measures in mice, as opposed to activation induced blood flow in humans) were associated with connectivity measures from cerebello-thalamo-cortical circuits (Ulug et al., 2011). In conclusion, these results reflect the human pathology and demonstrate that DYT1 mutant torsinA has similar effects in mice and humans despite the absence of overt motor pathology in the animal model.

Similarly, our results have stimulated further studies in mice, which overexpress mutant human torsinA (hMT1). These studies have elaborated on the cerebellar abnormalities found in our studies (Zhao et al., 2011): Using a combination of cytochrome oxidase histochemistry and 2-deoxyglucose autoradiography, high spatial resolution of metabolic activity can be obtained ex vivo. As in our studies, the authors demonstrated a shift of metabolic demand from the basal ganglia to the cerebellum in the hMT1 mouse (Zhao et al., 2011). Detailing on these findings, they were able to localize the abnormal metabolism to the Purkinje cell layer and the molecular layer of the cerebellum. In this mouse model, increased metabolic activity was also noted in the inferior olive, which sends excitatory input to cerebellar Purkinje cells via the climbing fiber pathway. Thus the altered Purkinje metabolism may be interpreted as a

consequence of olivary input change resulting in a change of firing properties of Purkinje cells (Zhao et al., 2011).

In another study the morphological cerebellar features were studied in *DYT1* ΔGAG knock-in (KI) mice (Zhang et al., 2011). These transgenic mice were found to have reduced lengths of primary dendrites and a decrease in the number of spines on the distal dendrites of Purkinje cells. In a further specification, mice with a knockout restricted to the DYT*1* gene only in Purkinje cells were created (to exclude the possibility of adaptive changes in other brain regions). In these mice similar alterations in Purkinje cell morphology were found, confirming the idea of inherent cerebellar pathology rather than adaptive changes as consequences of basal ganglia alteration.

The normal function of torsinA is a continued topic of discussion. TorsinA is expressed throughout the brain, but with particularly high levels in dopaminergic neurons of the substantia nigra and in the deep cerebellar nuclei (Shashidharan et al., 2000), and with regard to the temporal pattern in particular during phases of intense neurodevelopment (Siegert et al. 2005; Vasudevan et al., 2006; McCarthy et al., 2012). Thus, these regions are prone to be affected by mutant torsinA dysfunction, particularly during periods of synaptogenesis, such as during cerebral maturation in childhood or adolescence. The cerebellar distribution of torsinA during juvenile neurodevelopment has been further detailed (Puglisi et al., 2013), showing that this protein is highly expressed in cerebellar nuclei as well as in the cerebellar cortex. The expression on Purkinje cells was characterized by a striking involvement of the dendrites and spines, but other cerebellar cortical cells also expressed this protein. Moreover, specific analyses showed that afferent projections to the cerebellar cortex, in particular GABA-ergic and glutamergic synaptic contacts and glial cells also expressed high levels of torsinA (Puglisi et al., 2013). These findings highlight the broad functional involvement of the mutated protein in DYT1 dystonia, and reflect the molecular basis of the observed changes in cerebellar metabolism and function.

While the back-tansfer from “bedside-to-bench” may seem counterintuitive, it is important to note that accepted animal models of dystonia are sorely needed for drug screening purposes. In the current situation, where animal models either fail to reproduce abnormal movements, or fail to reflect human pathology, it is particularly meaningful when parallels of human pathology and animal pathology can be demonstrated. In this vein the analogies of findings detailed above, highlight an important step towards the development of a generally accepted animal model of dystonia.

Contrasting to the DYT1 genotype, relatively few studies have focused on the function of THAP1, where mutations are found in the DYT6 genotype. THAP1 was found to be transcribed in the cerebral cortex, cerebellum, striatum, substantia nigra, thalamus and spinal cord (Zhao et al., 2013); importantly the highest expression of THAP1 was found during the

early neurodevelopment in the cerebellum (Zhao et al., 2013). As with torsinA, cerebellar Purkinje cells expressed THAP1, but the protein was also found in cortical pyramidal cells, thalamic relay nuclei, medium spiny and cholinergic neurons in the striatum as well as in dopaminergic neurons in the substantia nigra. This observation lends further support to the concept of hereditary dystonia as a neurodevelopmental disorder involving the cerebellum as a major component of neurocircuitry. In addition, this expression is in line with our observation of a more pronounced involvement of the striatal dopaminergic system (cf. B.6.) in the DYT6 genotype.

The role of the cerebellum has increasingly been recognized during the period of this series of studies (partly supported by the impact of our studies). A major contribution to this discussion was the work by Neychev and colleagues (2008), who implemented a series of experimental animal models to show the functional interaction the cerebellum and basal ganglia in the expression of dystonic symptoms. It was only later, that a direct anatomical connection of these structures was described using retrograde transneuronal virus transport labeling (Bostan et al., 2010). The recent acceptance of models including cerebellar contributions to mechanisms of dystonia is illustrated by the increasing number of reviews, which have devoted the focus to cerebellar abnormalities in human dystonia (Jinnah and Hess 2006; Avazino and Abbruzzese 2012, Sadnicka et al. 2012, Filip et al. 2013)

C.1.b. Neurodevelopmental Abnormalities

The major contribution of this line of work to dystonia research is the conceptual framework of dystonia as neurodevelopmental circuit disorder (Carbon et al., 2004b, Ozelius et al., 2011). Our early finding of abnormal sensorimotor network connectivity in NM-DYT1 (Carbon et al. 2004b) has since been corroborated and expanded in independent human studies in sporadic focal dystonias (Blood et al. 2006, 2012; Bonilha et al., 2007, 2009; Delmaire et al. 2007, 2009) and in DYT6 dystonia (Cheng et al., 2012), which showed widespread changes throughout all elements of the sensorimotor circuits

Importantly, the suggestion of DYT1 dystonia as a neurodevelopmental disorder related to abnormalities of cytoskeletal dynamics, as posited in our studies (e.g. Carbon et al., 2004b), is increasingly supported (Nery et al., 2008) and has sparked research in animal models. In co-immune precipitation studies, Hewett and colleagues (2006) found an association between vimentin and torsinA in one complex including other cytoskeletal elements. Overexpression of mutant torsinA inhibited neurite extension in human neuroblastoma cells, with torsinA and vimentin immunoreactivity enriched in the perinuclear region and in cytoplasmic inclusions. The molecular underpinnings of neurodevelopmental effects of torsinA mutations have also been explored in a study on neurogenesis and neuronal

migration of the embryonic forebrain in a torsinA knock-out mouse (McCarthy et al., 2012), which showed subtle, but significant alterations in neuronal migration.
Recently, DYT4 dystonia has been linked to a missense mutation within the gene β-tubulin 4a, which codes for an autoregulatory domain of β-tubulin (Hersheson et al., 2012). Since tubulin is the fundamental element of microtubules, this finding provides yet another link for a role of the cytoskeleton in dystonia pathogenesis. Importantly, β-tubulin 4a is brain specific with highest expression in the cerebellum, followed by the putamen.

C.1.c. Parietal Abnormalities

Throughout all of our studies, we found abnormal metabolism and activation in the parietal cortex in the dystonias (Ghilardi et al., 2003; Carbon et al., 2004a, 2013, 2011, 2010). This area showed increased metabolism in manifesting as opposed to non-manifesting DYT1 and DYT6 mutation carriers (Carbon et al., 2004a). Moreover, this area showed the only overlapping metabolic abnormality in different hereditary dystonias (Carbon et al., 2013). A dysfunction of this region was also noted in the DYT1 genotype during repetitive reaching (Ghilardi et al., 2003) and during visual or motor sequence learning (Carbon et al., 2011). It is likely that both of these features are interrelated, even though metabolic values do not correlate with measures of learning performance. In a broader scope, it is rather likely that parietal abnormalities also contribute to those somatosensory integration deficits that have been repeatedly described in the dystonias (Tinazzi et al., 2009).

The posterior parietal cortex serves somatosensory integration (Xing and Andersen, 2000; Hsiao 2008) and the role of somatosensory perception and integration is increasingly recognized in dystonia (Konczak and Abbruzzese 2013; Quartarone and Hallett 2013). While a broader spectrum of somatosensory integration or perception abnormalities have been reported in sporadic focal dystonias (Tinazzi et al., 2009), deficient temporal discrimination has been reported in DYT1 dystonia (Fiorio et al., 2007). Affected and non-affected DYT1 mutation carriers showed a significantly reduced ability to temporally discriminate tactile-tactile or tactile-visual stimulation pairs. Moreover, affected DYT1 mutation carriers showed impaired temporal visual-visual discrimination, indicating a continuum of genotype to phenotype relation. The posterior parietal metabolic abnormalities as observed in our studies may give rise to somatosensory integration deficits, as this region has been shown to be active during sensory temporal order judgment tasks in healthy subjects (Nakashita et al., 2008; Lewandowska et al., 2010; Takahashi et al., 2013).

Parietal dysfunction in dystonia has also been demonstrated in other imaging studies (Elbert et al., 1998; Sanger et al., 2002; Butterworth et al., 2003; Dolberg et al., 2011; Dresel et al., 2011; Opavsky et al. 2011; Delnooz et al., 2012; Moore et al., 2012; Tinazzi et al., 2013), but

the integration of these findings into the concept of dystonia is an ongoing debate. Nevertheless, there are treatment approaches, at least for task induced focal dystonias addressing the somatosensory integration abnormalities in dystonia (e.g. Candia et al., 2003).

Moreover, linking the parietal abnormalities back to abnormal cerebellar function, widespread direct cerebellar-cortical connections have now been clearly demonstrated, and importantly so, these involve strong interactions with the posterior parietal cortex (Bostan, et al., 2013).

C.2. Neurochemical Changes in Dystonia

C.2.a. The Dopaminergic System

Our studies indicated that subtle changes in dopaminergic neurotransmission are present in DYT1 and more so in DYT6 dystonia. However, these were genotypic changes and unlikely to account for symptoms of DYT1 or DYT6 dystonia (Asanuma et al., 2005; Carbon et al., 2009). This lack of a symptomatic effect points to the causal potential of other neurotransmitter systems in DYT1 and DYT6 dystonia (such as e.g. cholinergic neurotransmission). Whether or not symptoms in sporadic dystonias are causally related to the dopaminergic systems remains to be determined in future research.

Our observations are in line with observations in animal models of DYT1 dystonia, which have found increased striatal dopa-decarboxylase (DOPAC) and homovanilineic acid (HVA) levels pointing to an increased dopamine turnover (Zhao et al., 2008; Page et al., 2010). By contrast, another study in a transgenic mouse model (with human mutant DYT1) showed a reduced amount of striatal D_2 receptor protein (Napolitano et al., 2010). Importantly, these animal studies pinpoint reasons for the reduced D_2 receptor availability that could not have been demonstrated in humans. Increased dopamine turnover can be demonstrated by amphetamine stimulation paradigms (e.g. Laruelle et al., 1996), but the use of amphetamine in dystonia is discouraged due to its potential to provoke or worsen dystonia (e.g. Thiel and Dressler, 1994; Priori et al., 1995).

It is important to recognize the genotypic or etiological differences of dystonias, as in other dystonias, such as DRD, tardive dystonia or levodopa induced dystonias, the dopaminergic system is clearly involved in symptom expression. By contrast, while dopaminergic modulation is therapeutically effective in these dystonias, a focus on other systems may be needed in other genotypes.

C.2.b. Perspective into Changes in Striatal Acetylcholine

Given that the dopaminergic system is unlikely to represent the key factor in at least DYT1 and DYT6 dystonia, the cholinergic system is a likely, but clearly under-researched contributor to mechanisms of disease in hereditary dystonias. One of the most effective therapies for these disorders, particularly during childhood and adolescence, is anticholinergic medication. Thus, abnormal cholinergic striatal function has been considered (Augood et al., 2002), but in-vivo evidence is limited for technical reasons. Moreover, cholinergic and dopaminergic striatal functions are so intricately interrelated, that both a direct defect within the cholinergic system or an indirect dysfunction of the cholinergic system due to (slight) dopaminergic dysfunction, or a combination of both could cause dystonia (Pisani et al., 2006). Long-term synaptic adaptations at cortico-striatal and thalamo-striatal synapses represent the basis of sensorimotor integration; and modulation of these depends on striatal cholinergic interneuron contacts with striatal medium spiny neurons (Calabresi et al., 2000). In turn, these cholinergic interneurons are dependent on nigrostriatal dopaminergic input (Aosaki et al., 1995). As a consequence, a loss of this dopaminergic input would lead to an increased excitability of cholinergic interneurons, with subsequent defective modulation of abnormal sensorimotor integration, as seen in dystonia. This intricate linking of cholinergic and dopaminergic abnormalities has been demonstrated in a series of current studies in a DYT1 mutant mouse model (Pisani et al. 2006, Martella et al., 2009; Sciamanna et al., 2012a,b).

C.3 Abnormal Network Activation in Dystonia

Our activation studies mainly focused on motor activation and sequence learning tasks. For these studies, the respective normal network topography was characterized first and network-performance relationships were calculated next (Carbon et al., 2003, 2010). Then, in a next step network expression was quantified in the disease state using prospective network calculation (Carbon et al. 2004d, 2010). Using this technique, we found consistently, that DYT1 and DYT6 mutation carriers do not show the same patterns of neuronal interregional connectivity that healthy controls showed (Carbon et al., 2010a, 2010b). This finding contrasts with findings in Parkinson's disease, in which despite the degenerative nature of the disease, normal network- performance relationships exist (Carbon et al., 2003). In Parkinson's disease these networks have even been demonstrated to be modulated with antiparkinsonian interventions (Carbon et al., 2003).

Decreased intracortical inhibition and abnormal cortical plasticity are consistent, shared features of sporadic and hereditary dystonias, but they have not yet been clearly linked to an underlying abnormality (Quartarone et al., 2006; Edwards et al., 2006). Although it is possible that these features relate to intracortical mircrocircuitry abnormalities, the widespread

changes found throughout all elements of the sensorimotor circuits in dystonia suggest a role of interregional neuronal relationships in the formation of these cortical abnormalities. In this vein, abnormal measures of structural connectivity have indeed also been demonstrated in sporadic dystonia (Delmaire et al., 2009; Bonilha et al., 2007, 2008; Blood et al., 2006). Whether or not the functional changes found in sporadic dystonia relate to altered structure-function relationships is elusive. As detailed above, for hereditary dystonias our line or work has shown a relationship of functional and structural networks.

Few external studies have addressed functional networks in dystonia: Moore and colleagues (2012) used motor studies with individuated finger control during f-MRI in task specific dystonia. They analyzed the data with a different network analysis technique, psychophysiologic interactions modeling, where seed volumes of interest are defined to identify individual task-dependent changes in functional connectivity between the defined volume of interest and the remaining brain. They found *in*creased functional connectivity of the posterior (sensorimotor) putamen with the cerebellum and SMC as well as *in*creased connectivity of the anterior (associative) putamen with SMA, the left PreMC, and left cerebellum. Similar results were reported in a resting state f-MRI study in writer's cramp (Mohammadi et al., 2012), which analyzed data with independent component analysis (i.e. hypothesis free covariation analysis, similar to our approach). In addition to increased basal ganglia connectivity, these authors reported reduced somatosensory cortex connectivity. Another resting-state f-MRI study in cervical dystonia (Delnooz et al., 2012) study found reduced connectivity within the sensorimotor network.

While it is possible that sporadic and hereditary dystonias show different abnormalities, in the same way as primary and secondary dystonia have been demonstrated to differ with regard to cortical plasticity (Kojovic et al., 2013), it is more likely, that differences in study design contribute strongly to seemingly contrasting results. F-MRI studies rely inherently on image subtraction techniques; thus baseline (group or task) differences often confound these studies.

In summary, our studies support the concept of dystonia as a motor circuitry disorder with a prominent role of cerebellar pathways in DYT1 and DYT6 dystonia (Carbon et al., 2004a; 2008c; Argyelan/Carbon et al., 2009). We propose that these dystonias result from a fundamental neurodevelopmental disturbance leading to anatomical/functional disconnection at the cerebellar level. Indeed, maldevelopment of cerebellar output pathways is a likely cause for the localized metabolic abnormalities seen in the resting state (Eidelberg et al., 1998; Trost et al., 2001; Carbon and Eidelberg, 2009) as well as those seen during motor and learning tasks (Ghilardi et al., 2003; Carbon et al. 2004d, 2008b, 2010a, 2011; Carbon and Eidelberg 2009).

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Erklärung

§ 4 Abs. 3 (k) der HabOMed
Habilitationsordnung der Medizinischen Fakultät
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Hiermit erkläre ich, dass

1. weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wird bzw. wurde,

2. die vorgelegte Habilitationsschrift ohne fremde Hilfe verfasst, die beschriebenen Ergebnisse selbst gewonnen sowie die verwendeten Hilfsmittel, die Zusammenarbeit mit anderen Wissenschaftlern/Wissenschaftlerinnen und mit technischen Hilfskräften sowie die verwendete Literatur vollständig in der Habilitationsschrift angegeben wurden,

3. mir die geltende Habilitationsordnung bekannt ist.

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Ort, Datum Unterschrift