How have functional and microstructural imaging contributed to the understanding of dystonia pathophysiology in DYT-1 gene carriers?

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Dystonias are a spectrum of movement disorders characterised by excessive involuntary muscle contractions, frequently leading to repetitive twisting movements and abnormal postures. They are classified along three axes: first by aetiology, into primary dystonia, secondary dystonia, dystonia-plus syndromes and paroxysmal dystonia; secondly by age of onset and thirdly by anatomical distribution, into generalised, focal, segmental and hemi-dystonia (1).

Various inheritance patterns have been identified in primary dystonia subtypes with genetic linkage studies. In addition, mapping has linked primary torsion dystonia to an autosomal dominantly inherited gene deletion with incomplete penetrance at dystonic locus DYT-1, known to result in mis-expression of a protein of unknown function called torsin A (2). Genetic loci for mixed-type, focal and segmental dystonias have also been mapped at DYT-6, DYT-7 and DYT-13 respectively (3).

The exact pathophysiology of primary dystonia is currently unknown. A number of cortical and subcortical pathological abnormalities have been identified, but whether these are primary or secondary changes, in addition to how they link together to cause disease, are unclear. This essay explores how functional and microstructural imaging have furthered the understanding of dystonia pathophysiology, particularly in DYT-1 gene carriers. I will first describe the common pathophysiological abnormalities discovered by older neurophysiological and transcranial magnetic stimulation techniques across dystonia subtypes. I will then examine the additional insight gained from positron emission topography (PET) metabolism imaging, diffusion tensor imaging (DTI) and radioligand binding imaging in DYT-1 carriers. Thirdly, using evidence from functional imaging studies, I will cover the theoretical therapeutic mechanism by which deep
brain stimulation (DBS) produces significant clinical benefits in primary torsion dystonia patients.

**Common Pathological Abnormalities from Electrophysiological and TMS Studies**

Basal ganglia dysfunction has been strongly associated with dystonia pathogenesis. Although no consistent structural abnormalities have been observed with MRI, meta-analyses have found disproportionate numbers of movement disorder patients with basal ganglia lesions presenting with secondary dystonia (36%) (4). The most common lesions occurred in the lentiform nucleus (putamen and globus pallidus). In addition, abnormal firing from the globus pallidus internus (GPI) were identified by microelectrode recordings during surgical treatments of dystonia, showing mean firing rates to be reduced (5) while discharge patterns appeared abnormally synchronous and irregularly grouped (6). Basal ganglia dysfunction was thought to disrupt its normal role in “scaling” and “focusing” movements. In addition, loss of basal ganglia inhibitory control of the thalamus has been hypothesised to contribute towards increased cortical excitability, known as the “release phenomenon” (7).

A number of studies have also implicated reduced intracortical inhibition as a cause of increased cortical excitability. Firstly, reduced short-interval intracortical inhibition measurements in writer’s cramp and torticollis patients were found in paired TMS studies (8). Secondly, application of bicuculline, a GABA antagonist, appeared to increase co-contraction of antagonistic muscles during simple wrist movements in monkeys (9). Thirdly, two-dimensional J-resolved MR spectroscopy measured reduced levels of GABA in the sensorimotor cortex and lentiform nuclei contralateral to the affected hand of patients with focal dystonia (10). Meanwhile, increased cortical excitability was demonstrated by measurements of motor evoked
potentials (MEP) following transcranial magnetic stimulation (TMS). Primary dystonia patients were found to consistently produce larger motor responses compared to controls when the same stimulus was applied, thus appearing to show a larger cortical output gain for the same input stimulus (11). Simultaneous increases in cortical excitability and decreases in intracortical inhibition appear to be significant pathological mechanisms.

Cortically, abnormal sensorimotor integration is also thought to contribute to dystonia pathogenesis. It is known that sensory inputs can modify the disorder: task-specific focal dystonias arise from repetition of highly-skilled tasks, during which high volumes of multiple sensory inputs are simultaneously integrated (12). In addition, decreasing the amount of sensory input with local anaesthetic (LA) injections near large afferent fibres were found to improve writer’s cramp (13), as did reduction of muscle afferent function with LAs into the forearm muscle motor points of writer’s cramp patients (14). Thirdly, sensory tricks, thought to alter the fusimotor drive, appear to reduce symptoms of focal dystonias (15). Fourthly, microelectrode mapping found increased receptive field (RF) sizes and significant overlaps between different RFs in the primary somatosensory cortices of overtrained dystonic monkeys (16). It is also thought that sensory deficits precede manifestation of dystonia (17). On the motor side, TMS has consistently shown distorted and displaced cortical motor maps for both affected and unaffected muscles of DYT-1 patients (18). Furthermore, magnetoencephalography (MEG) measurements on DYT-1 patients have shown disorganized sensorimotor representations to be a trait of all dystonia gene carriers, regardless of clinical status. Interestingly, while somatotopic disorganization correlating to the severity of dystonia was found in the primary somatosensory cortex representing the non-dystonic limb, disorganization in the cortex of the dystonic limb
appeared variable. This suggests that for dystonia to manifest clinically, a secondary process occurs in addition to a pre-programmed level of abnormality (19).

Abnormal plasticity has also been implicated by paired associated stimulation (PAS) and repeated TMS (rTMS) in dystonia pathophysiology. PAS applies paired stimuli to the cortex, one from an afferent neurone and one from TMS inducer, producing changes in synaptic excitability detectable as changes in MEP. As PAS produces effects that are consistent with spike timing dependent plasticity in animals as well as effects that can be abolished by drugs acting on NMDA receptors, it is accepted that the technique is a reliable probe of synaptic plasticity (20). PAS studies have been shown to have a greater effect on dystonia patients than in normal controls, suggesting aberrant neural plasticity play a role in the disorder (21).

Additionally, the use of “theta burst” rTMS has interestingly supported the idea of an abnormal neural substratum in all DYT-1 carriers proposed from MEG findings. Furthermore, rTMS appears to have unearthed protective and vulnerability factors for disease manifestation in DYT-1 carriers. When triplets of 50Hz stimulation are applied to the hand cortical area of control subjects every 200ms for 20 seconds, MEPs are usually suppressed for around twenty minutes (22). In affected DYT-1 patients, a longer than normal suppression was found. However, non-manifesting carriers of DYT-1 were found to show no response at all to rTMS (23). It appears that an increased response to plastic changes can lead to dystonia manifestation, while a reduced plasticity response has a protective element for DYT-1 carriers. However, does a protective response involving reduced synaptic plasticity have further implications for other brain functions, such as learning, for non-manifesting DYT-1 carriers?
Electrophysiological recordings and TMS studies have implicated roles for basal ganglia dysfunction, increased cortical excitability, reduced intracortical inhibition, abnormal sensorimotor integration and aberrant plasticity mechanisms in dystonia pathophysiology. The use of functional and structural imaging techniques have since translated these insights gained at the cellular level into an understanding of their effects at a cortical level.

**Abnormal Cortical Metabolic Activation At Rest**

While conventional imaging such as MRI and CT scans rarely detect gross structural abnormalities in primary dystonia patients, functional imaging studies using $^{18}$fluorodeoxyglucose (FDG) positron emission tomography (PET) have found a consistent pattern of increased cortical resting metabolism. Eidelberg first used principal component analysis to identify relative increases in metabolism in the cerebellum, parietal association area, supplementary motor area (SMA), posterior putamen and globus pallidus (24). Similar cortical activation patterns were found in two more independent cohorts of non-manifesting DYT-1 carriers (25,26). This abnormal metabolic activation network, termed torsion dystonia-related pattern (TDRP) was observed in clinically non-affected and affected carriers of DYT-1. Subsequent routine voxel-based univariate comparisons in a larger cohort of DYT-1 carriers confirmed the abnormal cortical metabolic network to be a distinctive trait of the DYT-1 gene absent from controls (27). Interestingly, TDRP was still present in affected DYT-1 patients even when sleep had been induced and dystonic movements had been suppressed (28). It appears that while TDRP is a trait of DYT-1 gene carriers, it does not necessarily lead to disease manifestation, supporting evidence from MEG and TMS studies for an abnormal neuronal substratum in all DYT-1 carriers.
Resting metabolic PET scans have also revealed the potential difference between pathophysioologies of dystonia subtypes. While studies with DYT-6 carriers found relative increases in metabolism similar to DYT-1 carriers in the pre-SMA and parietal association cortices, regional metabolism reductions specific to DYT-6 were found in the putamen, cerebellum and upper brainstem. It seems that while some resting metabolic abnormalities are genotype specific, others are common traits amongst different dystonia sub-types, confirming the clinical similarities between DYT-1 and DYT-6 (27). However, FDG PET imaging showed that commonly shared aspects of the TDRP network were not expressed in dopamine-responsive dystonia (DRD) patients (26). DRD patients appear to show a generally distinct metabolic topography, with relative increases in the dorsal midbrain, cerebellar vermis and a slight increase in SMA, with co-varying decreases in the putamen, lateral premotor and motor regions (29). The DRD pattern also differed from the Parkinson’s disease (PD) activation topography, surprising considering the appearance of Parkinsonian symptoms in the later course of DRD and DRD’s response to dopaminergic medication (29). It appears the pathophysiology of DRD might differ significantly from other forms of dystonia and PD.

In order to determine the primary and secondary changes within the abnormal metabolism activation patterns, spatial covariance analyses have been used to identify areas that appear phenotype-specific, and hence penetrance-related. Firstly, relative metabolic increases in pre-SMA and parietal association cortices, together with relative decreases in inferior cerebellum, brainstem and ventral thalamus are of greater magnitude in affected DYT-1 and DYT-6 patients compared to controls and non-affected gene carriers. Expression of this activation pattern, termed dystonia manifestation pattern (DYT-RP) are significantly increased in affected carriers
of both DYT-1 and DYT-6. Secondly, decreases in putamen activation appear significantly greater in magnitude in affected than non-affected DYT-6 gene carriers (30).

DYT-RP appears consistent with the “release phenomenon” hypothesis. Following evidence that cortical regional metabolism correlates with the degree of afferent input (31), the regions involved in DYT-RP appear compatible with electrophysiological measurements of reduced globus pallidus internus (GPI) functional output. A number of hyper-activated DYT-RP regions are output targets of the GPI, such as the pedunculopontine nucleus and ventrolateral thalamus.

The significant relation between clinical manifestation with abnormal activation of pre-SMA and parietal association areas reinforces the hypothesis that abnormal sensorimotor integration is a key cortical deficit in the pathogenesis of dystonia.

Resting PET scans of non-manifesting carriers of DYT-1 have also identified a potential stepwise deterioration of sensorimotor integration and a protective factor in dystonia patients. It is known that a particular polymorphism found at residue 216 of the torsin A codon has a significant relation to DYT-1 penetrance (32). DYT-1 carriers with the 216H allele appear less likely to manifest into dystonia than those without this polymorphism, hence attributing a possible “protective” role to 216H. When resting metabolism of non-manifesting DYT-1 carriers were measured, those without the “protective” allele showed relatively increased metabolism in the pre-SMA and parietal association cortex of an intermediate value between controls and affected patients. Meanwhile, the magnitude of dorsal premotor cortex (PMC) activation appeared similar to affected patients. Contrastingly, DYT-1 carriers with the “protective” allele showed normal metabolic activation in the pre-SMA and decreased activity compared to controls in the dorsal PMC and parietal association cortices (33). Resting metabolism PET imaging has hence shown that protective factors against dystonia manifestation exist for DYT-1 carriers. A
subtly different activation pattern for more vulnerable DYT-1 carriers suggests that development of abnormal sensorimotor integration occurs in a stepwise manner, via intermediate cortical abnormalities.

**Abnormal Cortical Metabolic Activation During Movement and Sequence Learning**

Abnormal metabolic activation patterns are also observed in cortices of non-affected and affected DYT-1 carriers when performing simple motor tasks. Similar to the resting TDRP, metabolism in SMA and PMC were found to be increased in non-affected DYT-1 carriers compared to controls (30). An additional reduction in poster-medial cerebellum activation, thought to be related to increased expression of torsin A in this area (34), is also observed. Affected DYT-1 patients showed even greater increases in cortical metabolic activations in PMC, SMA, parietal region and right anterior cerebellum during movement than non-affected carriers, with an additional activation of SMC found only in affected patients (35). This pattern again suggests an intermediate phenotype in non-manifesting gene carriers. Considering that non-affected DYT-1 carriers are able to produce simple movements, the changes in metabolic activation suggest that the cortico-striato-pallido-thalamic-cortical loop (CSPTC) has capacity within its neural substrate to compensate for underlying structural abnormalities.

However, a deficit is found when non-affected DYT-1 carriers perform more cognitively challenging tasks. During a motor sequence learning task, non-affected DYT-1 carriers correctly hit only 58% of targets compared to 80% in controls (36), despite the two groups showing similar movement initiation times, movement speeds and mean reaction times (37). Similar impairments were not found in affected or non-affected DYT-6 carriers, suggesting sequence learning deficiencies to be a distinctive subclinical trait of non-affected DYT-1 carriers.
When non-affected DYT-1 carriers performed a trial and error visually learned task, $H_2^{15}\text{O}$ PET imaging found that additional activation of the lateral cerebellum was required to achieve normal levels of learning (36). At the same time, the left anterior cingulated cortex, dorsal PMC and bilateral dorso-lateral prefrontal cortex (PFC), which in controls were regions only recruited during particularly difficult tasks (38), showed reduced metabolic activation. It appears that the cerebellum plays a compensatory role in non-affected DYT-1 carriers during cognitively difficult tasks, representing a striatal-cerebellar shift in processing mechanism (39). Secondly, sequence learning deficits found in DYT-1 carriers might be attributed to pre-existing structural abnormalities in the PFC, anterior cingulated cortex and dorsal PMC (40). In affected DYT-1 patients, increases in cerebellar activation similar to non-affected DYT-1 carriers could be observed but sequence learning remained impaired. It is possible that micro-structural abnormalities in cerebellar outflow tracts, such as known connections to the PFC (41), could prevent compensatory cerebellar activation from improving performance.

**Abnormal Microstructural Connections**

While the early mean age of onset of primary torsion dystonia appears to suggest that neurodevelopmental abnormalities underlie the condition (42), conventional MRI and CT imaging have been unable to identify consistent gross structural abnormalities. Instead, the microstructure of DYT-1 carriers have been investigated using diffusion tensor imaging (DTI), which employs fractional anisotropy as a measure of axonal integrity and coherence. Significant reductions in sensorimotor cortex subgyral white matter were found in all DYT-1 carriers, with further microstructural abnormalities around the superior cerebellar peduncle (43). These findings show that underlying structural abnormalities in the sensorimotor regions are present in dystonia pathophysiology. The implication of the cerebellar-thalamo-cortical pathway suggests
that microstructural abnormalities prevent effective cerebellar compensation in affected DYT-1 patients, leading to impairments of sequence learning despite increased cerebellar metabolic activation.

**Abnormal Dopaminergic Neurotransmission**

While the neurochemical basis of dystonia is unknown, abnormal dopaminergic neurotransmission is thought to be involved. Post-mortem examination of a DYT-1 patient had previously found reduced levels of dopamine in the rostral putamen and caudate (44). Significantly increased striatal dopamine metabolite (3,4-dihydroxyphenylacetic acid) to dopamine ratios were also found in the post-mortem of three DYT-1 patients (45). Using PET imaging with a radioligand called raclopride, reductions in dopamine binding to D2 receptors in caudate and putamen were shown in both non-manifesting and affected DYT-1 carriers (46). An expanded study identified further reduction of D2 binding amongst DYT-6 gene carriers. Furthermore, reduced dopaminergic transmission does not appear to simply be a subclinical trait of dystonic subtypes (30). Decreases in striatal D2 binding have been found to correlate significantly with increasing activation of the dystonia-manifestation related metabolic activation pattern in all DYT-1 and DYT-6 carriers (30). Abnormal dopamine neurotransmission appears to have an as yet undertermined on the penetrance of dystonia.

**Effect of Deep Brain Stimulation (DBS) on Dystonia**

Bilateral GPi DBS has produced remarkable and sustained improvement in some dystonia patients (47). While the exact therapeutic mechanism of this technique is unknown, functional imaging has shown that DBS reduces abnormal cortical metabolic activation in dystonia patients. Regional cortical blood flow (rCBF) imaging of a patient with “bilaterally on” DBS showed a
bilateral reduction in activity in the primary sensorimotor and prefrontal cortices when at rest and during a free selection joystick task. FDG PET imaging of idiopathic cervical dystonia patients found GPi DBS to be significantly associated with reduced resting activation of the pre-SMA, anterior cingulated cortex, inferior PFC and dorsolateral PFC (48). An extra reduction in metabolic activation at the anterior cerebellum was observed during a movement task. It appears that DBS normalises abnormally activated cortical motor regions (49). Further analysis of cortical activation found relative increases in ipsilateral sensorimotor activation areas, possibly also suggesting an increase in transcallosal inhibition following DBS (30). It should be noted that clinical improvement and coincident changes in cortical activation only appear over a matter of months, compared to a therapeutic benefit within hours in DBS for PD (50). This time course is compatible with the hypothesis that abnormal plasticity playing a role in dystonia pathogenesis. However, switching off DBS leads to worsening of symptoms within hours and subsequent return to maximum therapeutic benefit appears within hours of DBS being turned back on (51, 52). This would suggest that DBS works in part by forging a “new downstream network”, which is dependent upon electrical activity from continuous GPi DBS. Restarting DBS simply leads to reinstatement of a prior-constructed functional network (47).

While functional and microstructural imaging have produced significant insights into the cortical abnormalities found in dystonia, the pathophysiological mechanisms involved remain unclear. Any successful pathophysiological model of dystonia must unite all the implicated mechanisms from electrophysiological, TMS, microstructural and functional techniques, linking together abnormal cortical metabolism, microstructural abnormalities, impaired dopaminergic neurotransmission, basal ganglia dysfunction, altered cortical excitability, aberrant cortical plasticity and abnormal sensorimotor integration. In the meantime, functional imaging will no
doubt continue to contribute significantly to the understanding of dystonia. TDRP will prove to be a useful non-invasive marker in identifying non-affected carriers of dystonic genes for linkage studies. In addition, as in PD, changes in cortical metabolism can be useful in determining therapeutic success. It is hoped that understanding the pathophysiology of dystonia will one day allow development of curative treatments, beyond the limited symptomatic options currently available to thousands of patients around the world.
References

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