HHS Public Access

Author manuscript

Brain. Author manuscript; available in PMC 2015 June 03.

Published in final edited form as:

Brain. 2008 January ; 131(0 1): 146–154. doi:10.1093/brain/awm243.

Increased cerebellar activation during sequence learning in DYT1 carriers: an equiperformance study

Maren Carbon[1,2], **Maria Felice Ghilardi**[3], **Miklos Argyelan**[1], **Vijay Dhawan**[1,2], **Susan B. Bressman**[4], and **David Eidelberg**[1,2]

[1]Center for Neurosciences, The Feinstein Institute for Medical Research, North Shore-Long Island Jewish Health System

[2]Departments of Neurology and Medicine, North Shore University Hospital and New York University School of Medicine

[3]Department of Pharmacology and Physiology, City University of New York Medical School, New York, USA

[4]Beth Israel Medical Center, New York, New York, USA

Abstract

We have found that motor sequence learning and related brain activation is impaired in non-manifesting (nm) carriers of the DYT1deletion for dystonia. In the present study we used a trial-and-error sequence-learning task in conjunction with an equiperformance study design to identify the neural substrates that support sequence learning in nmDYT1 mutation carriers. Six nmDYT1 mutation carriers and six control subjects were scanned with $H_2{}^{15}O$PET during the performance of a trial-and-error guided, kinematically controlled motor sequence learning task and a matched motor execution task. Controls were matched for age and performance. PET data analysis was performed using statistical parametric mapping (SPM99). Although performing at matched levels, nmDYT1 mutation carriers over activated the lateral cerebellum and the right inferotemporal cortex relative to age-matched controls ($P < 0.001$). In contrast, they showed relative activation deficits in the dorsolateral prefrontal cortex bilaterally, as well as in the left anterior cingulate and the dorsal premotor cortex ($P < 0.001$). Prominent compensatory involvement of the cerebellum during target learning is consistent with our prior sequence-learning experiments in nmDYT1 mutation carriers. Contrasting to mutation carriers, normals used bilateral cerebellar activation in conjunction with a prominent prefrontal bilateralization only when confronted with a much higher task difficulty. nmDYT1 mutation carriers lack recruitment of these prefrontal regions that depend on modulation within the cortico-striato-pallido-thalamocortical (CSPTC) loops. Instead, they compensate solely using cerebellar activation. This observation is in keeping with recent evidence of impaired structure/function relationships within CSPTC networks in dystonia perhaps occurring on a neurodevelopmental basis. The inability to recruit the appropriate set of neocortical areas because of altered fronto-striatal connectivity may have led to the shift to cerebellar processing.

Correspondence to: Dr David Eidelberg, Center for Neurosciences, The Feinstein Institute for Medical Research, 350 Community Drive, Manhasset, New York 11030, USA david1@nshs.edu.

Keywords

sequence-learning; brain activation; PET; DYT1 dystonia

Introduction

Dystonia syndromes are characterized by excessive and sustained muscle contractions resulting in abnormal postures and involuntary movements. These syndromes are generally attributed to functional abnormalities in the cortico-striato-pallido-thalamocortical (CSPTC) loops and related pathways (Berardelli *et al*., 1998; Vitek, 2002). The most frequent genetic cause of primary dystonia is a heterozygous GAG deletion in the gene, DYT1, which results in the loss of a glutamic acid residue in the encoded protein, torsinA (Ozelius *et al*., 1997). As only 30% of mutation carriers clinically manifest dystonia (Bressman *et al*., 1994), studying non-manifesting DYT1 mutation carriers (nmDYT1) provides a unique opportunity to assess the effects of the genotype, without the confound of motor behavioural abnormalities associated with the phenotype (Eidelberg, 1998).

In previous positron emission tomography (PET) studies conducted in the resting state, we identified a consistent pattern of regional metabolic abnormalities that was present in manifesting as well as non-manifesting carriers of the DYT1 mutation (Eidelberg, 1998; Trošt *et al*., 2002). Mutation carriers exhibited relative increases in resting state glucose utilization in the putamen/globus pallidus, the supplementary motor area (SMA) and the lateral cerebellum (Eidelberg, 1998; Trošt *et al*., 2002). Because this genotype-related spatial covariance pattern included brain regions involved in motor planning and in the processing of sequential information, we proposed that sequence learning might be defective in carriers of the DYT1 deletion (Ghilardi *et al*., 2003). Indeed, we found that despite normal motor functioning, nmDYT1 mutation carriers demonstrated impaired sequence-learning performance. A comparable learning deficit has recently been reproduced in a DYT1 mutant mouse model in which age-dependent motor learning deficits were noted but only subtle signs of dystonia (Sharma *et al*., 2005). PET recording of brain activation responses in non-manifesting human carriers of the DYT1 deletion performing a serial reaction time sequence-learning task revealed abnormally increased activation of the left ventral prefrontal cortex, and of the right pre-SMA, occipital association cortex, and lateral cerebellum (Ghilardi *et al*., 2003). However, the relationship of these overactive regions to learning performance was not addressed in that study. Moreover, the presence of a sequence-learning deficit in nmDYT1 was limited to the experimental setting in which target presentation was continuous throughout the learning epoch.

In the present study we addressed these issues by utilizing a different sequence-learning task design to identify the neural substrates that support sequence learning in nmDYT1 mutation carriers. In contrast to the earlier activation study, in the current paradigm the sequence order was not revealed, and subjects had to discover the elements of the sequence by trial-and-error. This error-based learning design emphasizes the role of the cerebellum (Doya, 2000). Additionally, to identify the neural substrates that support sequence learning in nmDYT1 mutation carriers, we used an equiperformance learning design (Mentis *et al*.,

2003*b*). Control subject scans were chosen to match the nmDYT1 group performance level, thus facilitating the detection of compensatory brain activation. In this design, overactive areas do not account for performance differences, but illustrate the additional recruitment of neuronal resources during the learning process.

Methods

Subjects

Six right-handed nmDYT1 mutation carriers [five men and one woman; age 43.3 ± 9.5 years (mean ± SD); range: 33–57 years] were scanned with ^{15}O-labelled water ($H_2{}^{15}O$) and PET during the performance of a trial-and-error guided, motor sequence-learning task and a kinematically similar motor execution task (Ghilardi *et al*., 2000; Mentis *et al*., 2003*b*). These subjects were recruited, genotyped and neurologically examined through the Dystonia Clinical Research Center at Beth Israel Hospital in New York. They were all normal on neurological examination, did not show signs of dystonia, and did not have a history of dystonia.

We also studied six age-matched right-handed healthy volunteer subjects (three men and three women; age 42.7 ± 11.3 years; range: 29–57 years) who served as controls for the equiperformance comparisons with the mutation carriers. These subjects were selected from a larger group of 12 healthy volunteer subjects who performed these tasks during $H_2{}^{15}O$ PET imaging (Ghilardi *et al*., 2000). Those subjects selected as controls for the current study were matched for age with the nmDYT1 mutation carriers. In these age-matched control subjects, sequence-learning scans were selected from a total of 36 scans such that performance during each run was matched to that for each mutation carrier (Mentis *et al*., 2003b). Motor baseline scans for each control subject were chosen corresponding to the learning scans that were selected. Inclusion/exclusion criteria for mutation carriers and controls were otherwise as presented previously (Ghilardi *et al*., 2003). Written informed consent was obtained from all participants under protocols approved by the institutional review boards of the participating institutions.

Motor tasks

During scan acquisition, all subjects (nmDYT1 and controls) performed two runs of a trial-and-error-guided motor sequence-learning task (TSEQ) and a matched motor baseline task (CCW) with the dominant right hand. Both tasks have been described in detail previously (Ghilardi *et al*., 2000). In both tasks, subjects observed a computer screen, where radially arranged targets were displayed and had to be reached with the cursor of a hand-held mouse moving on a digitizing tablet. Similarly, in both tasks a tone at 1Hz intervals was presented to pace the reaching movements.

For TSEQ a new sequence of five or six targets had to be learned in each run. The sequence to be learned was not revealed to the subjects, but had to be guessed initially. In order to do so, subjects moved the cursor in self-initiated reaching movements to the target of their choice. Upon hitting the correct target subjects received feedback (targets that were correctly hit turned gray, while they remained transparent during incorrect hits). Via this explicit

feedback subjects gradually uncovered and memorized the sequence. In each novel sequence the number of targets equalled the number of targets on the screen. Each target was only visited once during the sequence and the sequence cycled through its veridical order throughout the duration of the scan.

In controls as well as in nmDYT1 mutation carriers, two subjects performed the task with five targets while four performed the task with six targets to be learned. The average rate of correctly hit targets was 58.3 ± 21.7 in the nmDYT1 and 58.2 ± 22.1 in the controls.

During the baseline motor task (CCW) subjects moved the cursor on the digitizing tablet with their right hand out and back from a central starting position to one of eight radial targets that were displayed in a predictable counterclockwise order. Subjects had to anticipate target appearance and to initiate the movement before the tone with a temporal window of ±100 ms. (A videoclip of this task can be seen at http://www.neuroscience-nslij.org.) Testing was done in separate trial blocks of 90 s. All subjects learned to perform the task in a training session conducted 1 or 2 days before testing. In the nmDYT1 group, the training session was also used to individually determine the longest sequence, which each DYT1 mutation carrier was able to learn in TSEQ. In the course of the training session, the number of targets per sequence was gradually increased from three to seven. The number of targets selected for testing during the PET recording was based upon each subject's performance during the last training session: this number was increased until subjects discovered and remembered the correct order of all targets within the first 60 s of the 90 s trial block.

During the scanning session each nmDYT1 mutation carrier performed TSEQ at this highest individual level. In contrast, controls were scanned while learning four, five and six targets in different runs. For group comparison, we then selected only those scans where normal performance matched nmDYT1 levels at the same number of targets.

To characterize performance during CCW and TSEQ, we computed several kinematic measurements for each movement as described previously (Ghilardi *et al*., 2000, 2003; Mentis *et al*., 2003*b*). To characterize performance in CCW, we calculated movement time, spatial error and the percentage of correct movements per trial. To characterize performance in TSEQ and CCW, we calculated the percentage of correct movements per learning block. A movement was categorized as correct when the directional error towards the target had <22° on either side of the appropriate target midpoint, and when the movement reversal occurred during the appropriate time window (100 ms around the pacing tone). Further details of the study design (Ghilardi *et al*., 2003) and the behavioural tasks (Ghilardi *et al*., 2000; Mentis *et al*., 2003*b*) have also been published previously.

Performance matching

For nmDYT1, the percentage of correct movements during TSEQ was 58.3 ± 21.7% (mean ± SE). For group comparison at equiperformance, a set of 12 control scans was selected from the healthy volunteer cohort matching the performance level of the DYT1 mutation carriers. The mean percentage of correct movements in the selected control group was 58.2 ± 22.1%.

During CCW, the mean percentage of correct movements was 97.8 ± 1.2% for nmDYT1 and 98.3 ± 0.9% for controls (Student's *t*-test; $P > 0.5$). Movement times during CCW were 389.3 ± 38.5 ms in nmDYT1 and 410.5 ± 48.7 ms in controls ($P > 0.3$). Spatial errors were 0.26 ± 0.09 cm in nmDYT1 and 0.27 ± 0.03 cm in controls ($P > 0.5$). Thus both groups were not only matched for learning performance, but also for motor performance levels.

Positron emission tomography

All subjects were scanned with $H_2{}^{15}O$ PET. They fasted overnight prior to scanning. PET imaging was performed using the GE Advance tomograph in 3D mode (Dhawan *et al*., 1998).

The details of the imaging procedures have been presented previously. In each run, relative regional cerebral blood flow (rCBF) was estimated using a modification of the slow bolus method (Silbersweig *et al*., 1993). Using this injection protocol, there was a time delay of ~17 s before onset of brain radioactivity, and the time from onset to peak count rate was 45–50 s. The timing of task initiation was individually adjusted so that the arrival of radioactivity occurred ~10 s after the start of each task. PET data acquisition began at the time of radioactivity arrival in the brain and continued for 80 s. The end of task thus coincided with the end of data acquisition. In this slow bolus $H_2{}^{15}O$ PET method, images reflect rCBF during the rising phase of the brain radioactivity, corresponding to the 2nd–8th cycles in our tasks. The interval between successive $H_2{}^{15}O$ administrations was 10 min to allow for the decay of radioactivity.

Image analysis

Scan preprocessing was performed using SPM 99 (Wellcome Department Cognitive Neurology, London, UK) implemented in Matlab (Mathworks, Sherborn, MA). The $H_2{}^{15}O$ PET scans from each subject were realigned using the mean image as a reference. All images were proportionally rescaled to a global CBF of 50 ml/min/dl and stereotaxically normalized into a standard anatomical space developed at the Montreal Neurological Institute (Collins *et al*., 1994). The images were smoothed with an isotropic Gaussian kernel (FWHM 10mm for all directions) to allow for interindividual gyral variation and to improve the signal-to-noise ratio.

The specific regions activated during TSEQ in the carriers of the DYT1 deletion and controls were identified using the multi-group, conditions and covariate option in SPM, with TSEQ and CCW representing the two conditions per subject and nmDYT1 and controls the two groups. Comparison of rCBF during the different conditions was performed by generating SPM{t} maps and defining the appropriate contrasts.

In all analyses, activations and regional correlations were considered significant at a threshold of $P \leq 0.05$, corrected for multiple comparisons. Additionally, we reported activations at $P \leq 0.001$ at peak voxel uncorrected ($T = 3.5$; for >80 contiguous voxels). Coordinates were reported in the standard anatomical space developed at the Montreal Neurological Institute.

All calculations were performed on PCs running Windows 2000. All additional statistical analyses were performed using JMP software (SAS Institute Inc., Cary, NC) for PC.

Results

Within-group analyses

Learning-related brain activation in controls—Analysis of learning-related brain activation (TSEQ>CCW) in controls revealed a large prefrontal cluster involving both hemispheres (Table 1, Fig. 1; green overlay). The activated region spanned the dorsal premotor cortex (dPMC), dorsolateral prefrontal cortex (DLPFC) (right>left) and anterior cingulate bilaterally. On the right side, this cluster extended into the ventral prefrontal cortex and into the pre-SMA. Additional task-specific activation was localized to the precuneus bilaterally, with left-sided predominance and to the right inferior parietal lobule.

Learning-related brain activation in nmDYT1mutation carriers—Analysis of learning-related brain activation (TSEQ>CCW) in nmDYT1 mutation carriers revealed bilateral activation of the dorsolateral prefrontal cortex (DLPFC), and of the right dPMC, extending into the anterior cingulate area (Table 2, Fig. 1; red overlay). Additional contributions were present bilaterally in parietal association regions and the cerebellum, and in right occipital and inferotemporal areas. A smaller cluster was also noted in the left ventral prefrontal cortex.

Between-group analysis

Decreased learning-related brain activation in nmDYT1mutation carriers compared to controls—Compared to controls, nmDYT1 mutation carriers showed markedly decreased activation of the bilateral DLPFC and the left dPMC (Table 3, Fig. 2A). Moreover, nmDYT1 mutation carriers deactivated the left cingulate cortex while this region was activated by controls during learning (Table 3, Fig. 2B).

Increased learning-related brain activation in nmDYT1 mutation carriers compared to controls—Increased task-specific activation relative to controls was detected in the left lateral cerebellum (Table 4, Fig. 3A). These increases were lateralized to the left at the specified thresholds for significance. However at slightly lower thresholds ($P < 0.005$), they were bilateral (Fig. 3A). Additionally, nmDYT1 mutation carriers activated the right inferotemporal cortex, while the controls deactivated this area (Table 4, Fig. 3B).

Discussion

In the present study we used an equiperformance design to identify compensatory brain activation during sequence learning in clinically unaffected DYT1 mutation carriers. Our results indicate that nmDYT1 mutation carriers utilize cerebellar activation to achieve successful sequence-learning at a moderate level of difficulty. The general pattern of TSEQ-related brain activation identified in controls and nmDYT1 includes the dPMC, pre-SMA, DLPFC, the cingulate and parietal areas. These areas have been previously implied in similar sequence-learning paradigms in healthy subjects (Sakai *et al*., 2002; Mentis *et al*., 2003*a*, *b*; Carbon *et al*., 2003; Halsband and Lange, 2006). With increasing task difficulty,

healthy subjects displayed prominent bilateralization of prefrontal and parietal learning-related activation, as well as activation of the cerebellum (Mentis *et al*., 2003*a*). In contrast, nmDYT1 did not exhibit the former activation responses during learning, but only activated the lateral cerebellum. Surprisingly, despite performing at only a moderate level of difficulty, and being matched to equally performing controls, nmDYT1 showed deficient activation of the bilateral DLPFC and left PMC. Moreover, in contrast to controls, nmDYT1 deactivated the cingulate region.

Our data support the notion that cerebellar activation in nmDYT1 is a compensatory mechanism to improve learning performance. This is consistent with our prior sequence-learning experiments in nmDYT1 (Ghilardi *et al*., 2003). However, since in our prior experiments nmDYT1 and healthy controls were not matched for performance, the functional role of the cerebellum could not be specified. Overactivation of the cerebellum in our earlier study could have been either compensatory or pathological. Resolving this question is important because the cerebellum has been implicated as a key structure involved in expression of dystonia (LeDoux *et al*., 1998; LeDoux and Brady, 2003; Raike *et al*., 2005; Jinnah and Hess, 2006). Moreover, cerebellar hypermetabolism is part of the abnormal metabolic brain network associated with the DYT1 genotype (Eidelberg, 1998; Trošt *et al*., 2002; Carbon *et al*., 2004*b*). The cerebellum and the basal ganglia are thought to have complementary roles in sequence-learning (Hikosaka *et al*., 1999; Doya, 2000; Ohyama *et al*., 2003). Cerebellar learning results from error-based feed-forward control (Ohyama *et al*., 2003), while the basal ganglia are specialized in reinforcement-based learning involving positive feedback (Graybiel, 2005). Additionally, the cerebellum is engaged in the earliest phases of sequence learning, with the cortex being activated before the dentate nucleus, while the striatum supports memory storage of sequences (Doyon *et al*., 2003). Our short trial-and-error-based task design emphasized the cerebellar role in the learning process. Nevertheless, striatal activation has been demonstrated in controls, possibly due to the presence of feedback acting as implicit reward (Carbon *et al*., 2003). In the nmDYT1 mutation carriers, resting state metabolic abnormalities in the basal ganglia (Eidelberg, 1998; Trošt *et al*., 2002), may have interfered with learning-related activation in this region, necessitating compensatory shifts to the cerebellum. Although DYT1 mutation carriers also display abnormal resting metabolism (Trošt *et al*., 2002) and abnormal motor activation (Ghilardi *et al*., 2003) in the lateral cerebellum, the functional shift that was observed during sequence learning was to cerebellar regions without metabolic abnormalities in the resting state or during simple motor execution.

Contrary to PD patients, who are likely to use physiological pathways throughout the disease (Carbon *et al*., 2003), nmDYT1 mutation carriers lack recruitment of prefrontal regions and compensate solely using cerebellar activation. This observation is in keeping with recent evidence of impaired structure/function relationships within CSPTC networks in dystonia (Vitek, 2002; Carbon *et al*., 2004*a*, *d*) perhaps occurring on a neurodevelopmental basis (Carbon *et al*., 2004*d*; Goodchild *et al*., 2005; Bahn *et al*., 2006; Hewett *et al*., 2006; Vasudevan *et al*., 2006). The inability to recruit the appropriate set of neocortical areas (see below) because of altered frontostriatal connectivity may have led to the shift to cerebellar processing. We note that in this case, compensatory brain activation occurs in a region that

may also mediate the clinical manifestations of disease (LeDoux *et al*., 1998; LeDoux and Brady, 2003; Raike *et al*., 2005; Jinnah and Hess, 2006). Indeed, compensatory brain activation can be viewed as a normal sign of adaptive cerebral plasticity (Johansen-Berg *et al*., 2002; Ptito *et al*., 2005; Desmurget *et al*., 2007). However, an association between abnormal neuroplasticity and symptoms has long been recognized in focal dystonia (Byl *et al*., 1996; Quartarone *et al*., 2006; Weise *et al*., 2006). If cerebellar overactivation is viewed as a maladaptive neuroplastic response in nmDYT1 carriers, a similar abnormal relationship may underlie the appearance of symptoms in affecteds with DYT1 dystonia.

Surprisingly, we also found that nmDYT1 mutation carriers activated the right inferotemporal area, while controls de-activated this area. The latter observation is in line with earlier findings in healthy controls performing a similar sequence-learning task (Sakai *et al*., 2002). Although a role of the ventral visual stream in motor control appears contradictory to the distinct functions of the dorsal and ventral visual stream (Goodale and Milner, 1992), Fogassi and Luppino (2005) have recently emphasized the role of parietoinferotemporal connections in motor control. The inferotemporal cortex receives strong input from the inferior parietal lobule in the primate (Tanaka, 1996; Zhong and Rockland, 2003). This conjoined input is implied in retrieving the semantic properties of objects during a motor act (Fogassi and Luppino, 2005). It is possible that activation of the inferotemporal cortex during sequence learning in nmDYT1 mutation carriers indicated the use of alternate learning strategies that rely on visual object properties (rather than on or in addition to sensory properties). Although the motor baseline task and the learning task are matched for sensory input during target presentation, subtle differences in the sensory feedback that are not important in controls may necessitate the use of such an alternate learning strategy in the face of sensory processing deficits in the DYT1 mutation carrier state (Sakai *et al*., 2002; Fiorio *et al*., 2007).

It is striking that all areas showing reduced learning-related activation in nmDYT1 (DLPFC, anterior cingulate and dPMC) are cortical elements of the major CSPTC loops. DLPFC in conjunction with the anterior cingulate is typically activated when healthy subjects learn new motor sequences (Jenkins *et al*., 1994; Jueptner *et al*., 1997*a*, *b*; Ghilardi *et al*., 2000; Nakamura *et al*., 2001; Carbon *et al*., 2003; Mentis *et al*., 2003*a*; for review see Ashe *et al*., 2006). This activation with right-sided predominance is typically attributed to the aspect of spatial working memory (Wager and Smith, 2003; Curtis, 2006; Halsband and Lange, 2006; Muller and Knight, 2006) as well as to the aspect of attention to action (Jueptner *et al*., 1997*a*, *b*). Surprisingly, relatively decreased DLPFC activation in nmDYT1 was not found in our earlier study (Ghilardi *et al*., 2003). This discrepancy may have resulted from the increased working memory load associated with trial-and-error-based target detection as compared with the earlier serial reaction time task, especially given that DLPFC activation is typically related to working memory load (Rypma and D'Esposito, 1999; Rypma *et al*., 2002; Lewis *et al*., 2004; Leung *et al*., 2007). It is possible that the inadequate prefrontal response to task demands in the nmDYT1 mutation carriers is caused by an impairment of frontostriatal connectivity. Striatal modulation of the cortical response is critical for task performance (Carbon *et al*., 2004*c*) and may be impaired in nmDYT1 carriers, who display

abnormal resting striatal metabolism (Eidelberg, 1998; Trošt *et al*., 2002) and reduced D2-receptor binding (Asanuma *et al*., 2005).

Consistent with our previous study (Ghilardi *et al*., 2003), we found that nmDYT1 carriers de-activated the dorsal anterior cingulate (BA 32) during sequence learning, while healthy controls activated this area. Anterior cingulate (ACC) activation in conjunction with lateral prefrontal activation has been repeatedly observed during sequence learning in health (Jueptner *et al*., 1997a, b; Ghilardi *et al*., 2000; Nakamura *et al*., 2001; Sakai *et al*., 2002; Carbon *et al*., 2003). However, ACC activation is not specific to sequence learning, but occurs during a wide array of cognitive tasks. Therefore caudal ACC activation has been proposed to reflect action monitoring either via response conflict monitoring (Barch *et al*., 2001; Gehring and Fencsik, 2001; di Pellegrino *et al*., 2007) or via error detection (Gehring and Knight, 2000; Swick and Turken, 2002; Mars *et al*., 2005; van Veen and Carter, 2006). In particular, it has been proposed that intact prefrontal-ACC interaction is crucial for proper action monitoring (Gehring and Knight, 2000; Swick and Turken, 2002; Markela-Lerenc *et al*., 2004; Dias *et al*., 2006). Abnormal activation was present in both of these areas in the DYT1 mutation carriers, suggesting the possibility of disturbed action monitoring processes.

The attenuation of learning-related activations in the PMC may relate to the previously observed overactivation of this region in DYT1 mutation carriers performing the motor execution task without learning (Ghilardi *et al*., 2003). Nonetheless, in this region there was no group difference in activation during the performance of the motor task. It is conceivable that a ceiling in the local brain activation may have been reached during movement, thus not permitting further activation in response to the additional challenge of motor sequence-learning. Alternatively, impaired PMC activation during learning may be secondary to functional abnormalities in the basal ganglia. Because of the strong anatomical relationship between these structures (Nakamura *et al*., 2001; Carbon *et al*., 2003), the results may also be consistent with a disruption of cortico-striatal functional connectivity in carriers of the DYT1 deletion.

We recognize that the number of subjects in the DYT1 carrier and control groups is small and that the results may not necessarily be generalized to other cohorts. That said, there was no evidence of a difference between the learning-related activation responses observed in the six healthy volunteers used for equiperformance comparison with the nmDYT1 carriers and those recorded in the remaining six normal subjects who comprised our original cohort (Ghilardi *et al*., 2000). Likewise, additionally the gender distribution was not strictly matched across the carrier and control groups ($P = 0.09$, chi-square test). We note that in the original TSEQ study of 12 healthy volunteers, learning-related activation in the precuneus region was significantly greater ($P < 0.001$) for male versus female subjects. Nonetheless, this region was not among those that discriminated DYT1 carriers from controls during learning. Moreover, there were no identifiable gender differences in any of the regions that were found to distinguish the two groups at equiperformance. Future studies will help determine whether similar changes in regional brain function are evident in affected DYT1 carriers performing this and related sequence-learning tasks.

Conclusion

The presence of a stable different learning topography in non-manifesting DYT1 mutation carriers raises the possibility of functional reorganization of fronto-striatal pathways in these subjects, perhaps on a genetic or developmental basis. Indeed, metabolic changes in the basal ganglia may lead to the shift from striatal to cerebellar processing as a feature of the DYT1 mutation carrier state. The clinical presentation of dystonia in DYT1 mutation carriers may reflect a limitation of the compensatory increased activation responses related to motor control that occur with this genotype.

Acknowledgements

This work was supported by the National Institutes of Health (NIH RO1 NS 047668 (D.E.)). The authors would like to thank Dr Thomas Chaly for radiochemistry support, Loreta Quartarolo and Deborah Raymond for support in study coordination and patient recruitment, and Nathaniel Brown and Toni Flanagan for editorial assistance.

Abbreviations

CSPTC	cortico-striato-pallido-thalamocortical
SMA	supplementary motor area

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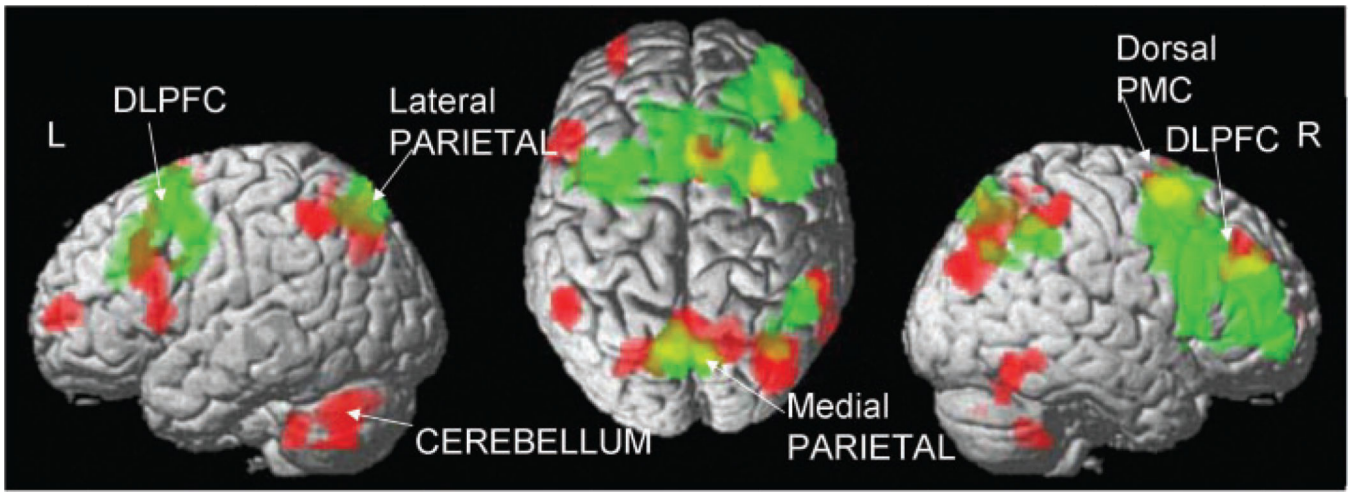

Fig. 1.

Activation responses during the trial-and-error-based sequence-learning task in nmDYT1 mutation carriers and controls (see text; Tables1 and 2).The surface rendering of the statistical map (SPM99 canonical template) reflects sequence-learning-related activation (TSEQ>CCW) specific to the six non-manifesting DYT1 mutation carriers (red) and specific to the six controls (green) as well as areas of overlapping activation (yellow). Sequence-learning activation responses in both groups were localized to the dorsolateral prefrontal cortex (DLPFC), dorsal premotor cortex (PMC) and to the medial and lateral parietal association cortices. Significant cerebellar activation responses were present in theDYT1group only.

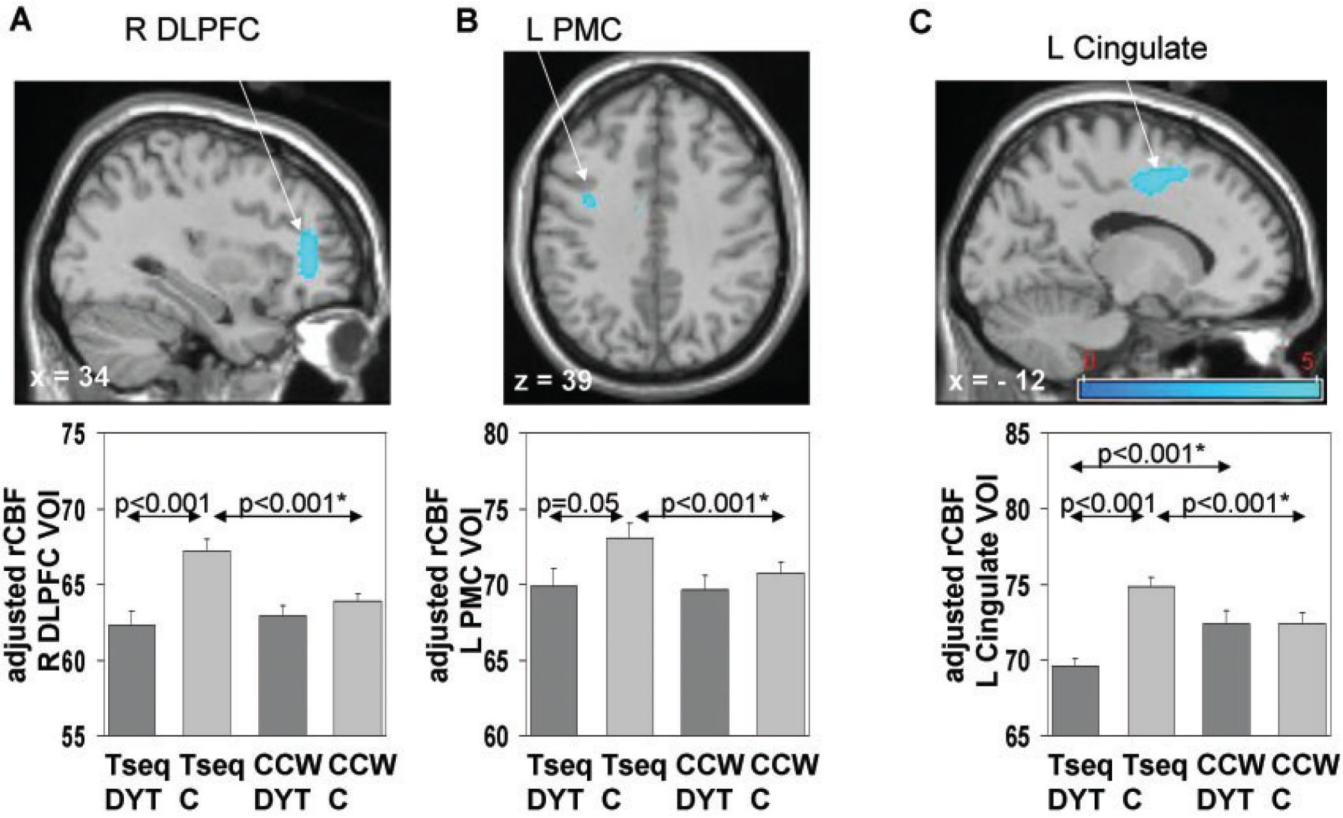

Fig. 2.

Brain regions in which learning-related activation responses during trial-and-error-based sequence-learning were reduced in nmDYT1 carriers relative to controls (Table 3, see text): SPM{t} maps (top panel) were superimposed on a single-subject MRI T1 template (*x*, *y*, *z* coordinates in MNI space indicate the position of the slice). Bar diagrams (bottom) illustrate adjusted regional cerebral blood flow (rCBF) during sequence learning (TSEQ) and during the motor reference task (CCW) in the respective cluster/VOI (mean ± SE) in the DYT1mutation carriers (dark gray) and controls (light gray). Decreased learning-related activation was present in the DLPFC (**A**) as well as in the left dPMC (**B**) with significant activation in controls, but not in carriers of the DYT1mutation. Additionally, nmDYT1s deactivated the anterior cingulate cortex (B), while controls activated this region. Significant contrasts of *post hoc* VOI comparisons are displayed (*P*-values of *paired T*-tests marked with asterisk). Brain activation during the baseline motor task was comparable across groups in all displayed regions ($P > 0.2$). (The colour stripe represents *T*values thresholded at 3.5, $P < 0.001$.)

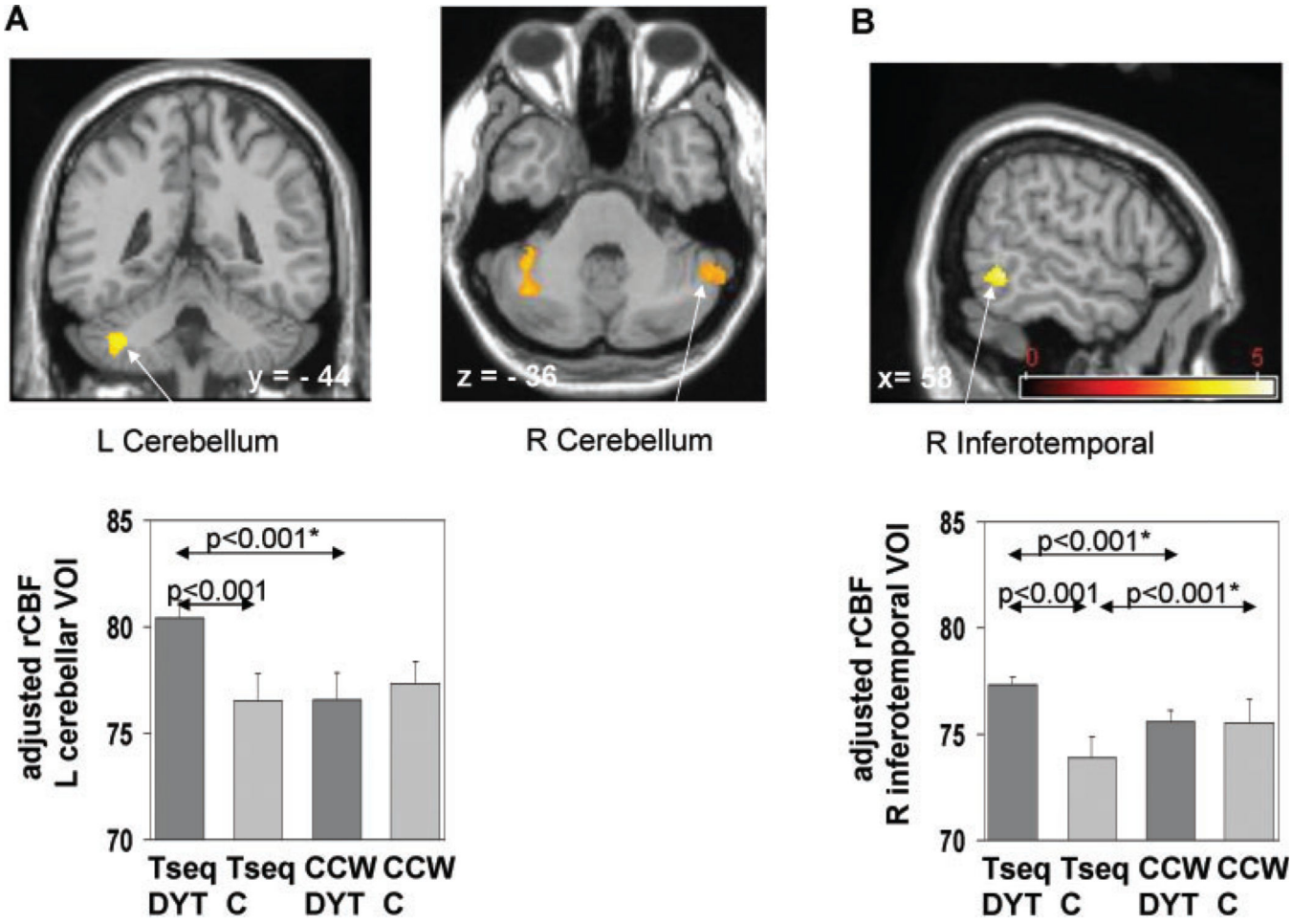

Fig. 3.

Brain regions in which learning-related activation responses during trial-and-error-based sequence-learning were increased in DYT1 carriers relative to controls (Table 4; see text): SPM{t}maps (top panel) were superimposed on a single-subject MRI T1 template (*x*, *y*, *z* coordinates in MNI space indicate the position of the slice). Bar diagrams (bottom) illustrate adjusted regional cerebral blood flow (rCBF) during sequence learning (TSEQ) and during the motor reference task (CCW) in the respective cluster/VOI (mean ± SE) in the DYT1 mutation carriers (dark gray) and controls (light gray). Increased learning-related activation was present in the left cerebellar cortex and to a lesser degree in the right-sided homologue (**A**) as well as in the right inferotemporal cortex (**B**). Brain activation during the baseline motor task was comparable across groups in all displayed regions ($P > 0.2$). *P*-values refer to *post hoc* VOI testing using unpaired and paired (asterisk) *T*-tests. (The colour stripe for the left and right column represents *T* values thresholded at 3.5, $P < 0.001$, *T* values for the middle column were thresholded at 3.0, $P < 0.005$.)

Table 1

Brain regions with significant increases in TSEQ related brain activation (TSEQ > CCW) in controls (see Figure 1)

Brain Region	Coordinates[a]				Cluster extent ke
	Z_{max}	*x*	*y*	*z*	
R DLPFC (BA 46)	5.2	30	40	18	8233
R DLPFC (BA 9)	4.2	44	2	46	
L DLPFC (BA 9)	4.0	−34	−2	40	
R dPMC (BA 6)	4.1	46	10	34	
L dPMC (BA 6)	4.2	−26	14	64	
R preSMA (BA 6)	4.0	12	8	60	
R frontal eye field (BA 8)	4.0	48	22	52	
R ventral PFC (BA 45)	3.9	64	18	2	
R ACC (BA 32)	3.5	10	26	40	
L ACC (BA 32)	3.8	−2	26	36	
R precuneus (BA 7)	4.9	2	−78	58	805
L precuneus (BA 7)	4.8	−10	−70	54	
R inferior parietal lobule (BA 40)	4.1	50	−46	40	430

For large clusters (>500 voxels) the regional distribution is illustrated using the localization of the peak voxel (left-most entry to the left column) and the localization of the submaxima of the respective cluster (right-most entry to the left column) (Significant at $P < 0.05$ corrected for multiple comparisons).

DLPFC = dorsolateral prefrontal cortex; BA = Brodmann Area; dPMC = dorsal premotor cortex; ACC = anterior cingulate cortex.

[a]Montreal Neurological Institute (MNI) standard space.

Table 2

Brain regions with significant increases in TSEQ related brain activation (TSEQ > CCW) in nmDYT1 (see Figure 1)

Brain region	Coordinates[a]				Cluster extent ke
	Z_{max}	*x*	*y*	*z*	
R lateral cerebellar cortex**	3.6	40	−50	−46	84
L lateral cerebellar cortex*, lobulus VI	4.8	−36	−64	−34	984
L lateral cerebellar cortex, crus I	4.0	−48	−60	−50	
R inferotemporal (BA 37)*	4.7	66	−62	−16	251
L ventral prefrontal (BA 10)**	3.6	−32	58	10	132
L DLPFC (BA 9, 44–46)*	4.0	−56	22	22	330
R DLPFC (BA 9/46)*	3.9	44	34	28	240
R angular (BA 39)*	5.1	32	−74	30	802
R occipital (BA 19)	4.6	38	−78	26	
R superior parietal (BA 7)	4.5	36	−72	42	
L precuneus (BA 7)*	4.8	−14	−74	44	1153
R precuneus (BA 7)	4.1	8	−66	50	
L inferior parietal lobule (BA 40)**	4.7	−56	−52	50	206
R inferior parietal lobule (BA 40)*	4.3	60	−42	54	257
R dorsal PMC (BA 6)*	4.8	30	8	62	232
R pre-SMA (BA 6)*	3.9	4	12	62	291

DLPFC = dorsolateral prefrontal cortex; BA = Brodmann Area; PMC = premotor cortex; SMA = supplementary motor area.

[a] Montreal Neurological Institute (MNI) standard space.

* $P < 0.05$ corrected for multiple comparisons,

** $P < 0.001$ uncorrected.

Table 3

Brain regions with relatively decreased activation in TSEQ in nmDYT1 carriers compared to controls

Brain region	Coordinates[a]				Cluster extent ke
	Z_{max}	*x*	*y*	*z*	
R DLPFC (BA 10/46)*	4.0	34	40	2	315
L DLPFC (BA 9/32)**	3.8	−18	38	30	84
L PMC (BA 6)**	3.8	−34	−2	40	81
L dorsal anterior cingulate (BA 32)*	4.0	−14	0	46	490

DLPFC = dorsolateral prefrontal cortex; BA = Brodmann Area; PMC = premotor cortex.

[a]Montreal Neurological Institute (MNI) standard space.

*$P < 0.05$ corrected for multiple comparisons,

**$P < 0.001$ uncorrected.

Table 4

Brain regions with relatively increased activation in TSEQ in nmDYT1 carriers

Brain region	Coordinates[a]				Cluster extent ke
	Z_{max}	*x*	*y*	*z*	
L lateral cerebellar cortex, lobulus VI	3.5	−34	−44	−42	97
R inferotemporal area (BA 37)	3.9	58	−52	−8	87

BA = Brodmann Area.

($P < 0.001$, uncorrected).

[a]Montreal Neurological Institute (MNI) standard space.