Defective temporal processing of sensory stimuli in *DYT1* mutation carriers: a new endophenotype of dystonia?

Mirta Fiorio,[1] Mattia Gambarin,[1] Enza Maria Valente,[3] Paolo Liberini,[5] Mario Loi,[6] Giovanni Cossu,[6] Giuseppe Moretto,[2] Kailash P. Bhatia,[8] Giovanni Defazio,[7] Salvatore M. Aglioti,[4] Antonio Fiaschi[1] and Michele Tinazzi[1,2]

[1]Department of Neurological and Vision Sciences, Section of Rehabilitative Neurology, University of Verona, [2]Neurology Unit, Borgo Trento Hospital, Verona, [3]IRCCS CSS, Mendel Institute, [4]Department of Psychology, University of Rome 'La Sapienza' and Neuropsychology Research Centre, IRCCS 'Fondazione Santa Lucia', Rome, [5]Neurology Clinic, Brescia, [6]Neuropsychiatry Department, G. Brotzu Hospital, Cagliari, [7]Institute of Neurology, University of Bari, Italy and [8]Sobell Department of Motor Neuroscience and Movement Disorders and Institute of Neurology, London, UK

Correspondence to: Mirta Fiorio, Department of Neurological and Vision Sciences, Section of Rehabilitative Neurology, University of Verona, Via Casorati 43, 37131 Verona, Italy
E-mail: mirta.fiorio@medicina.univr.it

DYT1 primary torsion dystonia is an autosomal dominant movement disorder due to a 3-bp GAG deletion in the *TOR1A* gene, which becomes manifest in only 30–40% of mutation carriers. Investigating the factors regulating this reduced penetrance might add new insight into the mechanisms underlying the disease. The pathophysiology of dystonia has been related to basal ganglia dysfunctions that lead to the most prominent motor symptoms. However, subclinical sensory deficits have also been reported, particularly in adult-onset focal dystonia. Sensory abnormalities in different forms of sporadic dystonia have been revealed by using a psychophysical method, namely, the temporal discrimination threshold (TDT), quantified as the shortest time interval at which the two stimuli are perceived as separate. Little or no information about the presence of sensory abnormalities in *DYT1* gene manifesting and non-manifesting carriers is available. With the aim of disclosing possible associations between sensory deficits and the *DYT1* mutation, we assessed TDTs of DYT1 manifesting patients (*n* = 9); DYT1 non-manifesting relatives (*n* = 11); non-carrier relatives (*n* = 9); external control subjects (*n* = 11). Pairs of tactile, visual or visuo-tactile stimuli were delivered in blocked, counterbalanced order. Intervals between stimuli increased from 0 to 400 ms (in 10 ms steps). On each trial, subjects had to report whether stimuli occurred simultaneously or asynchronously. We measured the first out of three consecutive inter-stimulus intervals at which subjects recognized the two stimuli as temporally separated (TDT) and the first of three consecutive intervals at which they also reported correctly which stimulus in the pair preceded (or followed) the other temporal order judgment (TOJ). Results showed higher tactile and visuo-tactile TDTs and TOJs in DYT1 carriers, both manifesting and non-manifesting, compared with non-carrier relatives and with external control subjects (for all comparisons, *P* < 0.039). This finding indicates that the *DYT1* mutation determines subclinical sensory alterations, which could be disclosed by a psychophysical task. Moreover, these results have the notable implication that sensory deficits in dystonia are not a mere consequence of abnormal movements, but they may even occur before overt clinical manifestations, representing a subclinical phenotype in *DYT1* mutation carriers.

Introduction

Primary torsion dystonia (PTD) is a movement disorder characterized by prolonged muscular contractions causing abnormal torsion movements and sustained postures (Fahn *et al.*, 1998; Bressman, 1998). The clinical spectrum is wide, ranging from early-onset dystonia, which usually becomes generalized, to late-onset, focal forms.

So far, only one PTD gene (*TOR1A/DYT1*) has been cloned (Ozelius *et al.*, 1997). A unique heterozygous GAG deletion is responsible for the majority of PTD cases with early-onset in a limb and rapid generalization (Ozelius *et al.*, 1997; Valente *et al.*, 1998; Nemeth, 2002). The *DYT1* mutation is inherited with autosomal dominant inheritance and markedly reduced penetrance: in fact, only 30–40% of mutation carriers develop dystonia during their life (Ozelius *et al.*, 1997; Saunders-Pullman *et al.*, 2004). Genetic or environmental factors regulating penetrance are still largely unknown. Investigating possible subclinical similarities and differences between manifesting and non-manifesting DYT1 carriers might help characterize the factors influencing penetrance and perform genotype–phenotype correlates.

Albeit still largely unknown, the pathophysiology of PTD has been related to basal ganglia dysfunctions leading not only to the most prominent motor symptoms, but also to subclinical sensory deficits. Sensory feedback is crucial for driving motor outputs. Thus, although at first counter-intuitive, the impairment of sensory functions may play a fundamental role in the pathophysiology of dystonia (Hallett, 1995; Abbruzzese *et al.*, 2001; Tinazzi *et al.*, 2003). Indeed, defective sensory functions have been demonstrated in several forms of dystonia. Temporal discrimination is a basic aspect of somatosensory processing, essential for a number of functions including kinaesthesia, graphaesthesia, vibratory sense and stereognosis. Assessment of this function has been carried out by using the psychophysical procedure of computing somatosensory temporal discrimination threshold (TDT), defined as the shortest time interval at which two stimuli are perceived as separate. Studies converge to indicate that thresholds were much higher in patients with generalized, cervical and focal-hand dystonia than in control subjects (Bara-Jimenez *et al.*, 2000; Sanger *et al.*, 2001;

Aglioti *et al.*, 2003; Fiorio *et al.*, 2003; Tinazzi *et al.*, 2004). These deficits have been interpreted in light of the relationship between dystonia and dysfunctions of basal ganglia that are implicated not only in motor control, but also in temporal processing (Lacruz *et al.*, 1991; Artieda *et al.*, 1992; Harrington *et al.*, 1998a). Interestingly, we observed sensory deficits also in the unaffected hand of patients with unilateral focal-hand dystonia (Fiorio *et al.*, 2003). This would suggest that sensory abnormalities occur independently from the localization of the motor symptoms and may occur before overt manifestation of dystonia (Meunier *et al.*, 2001; Fiorio *et al.*, 2003; Garraux *et al.*, 2004).

The main aim of the present study is to reveal whether any sensory dysfunctions in DYT1 dystonia may be related to the abnormal genetic substrate and thus represent a sensory endophenotypic trait of disease. To this purpose, we applied the temporal discrimination paradigm (Fiorio *et al.*, 2003) in manifesting and non-manifesting DYT1 carriers, and in non-carrier relatives as well as in external control subjects.

Material and methods
Subjects

We recruited a total of 40 subjects subdivided in the following groups: (i) DYT1 manifesting patients ($n = 9$); (ii) DYT1 non-manifesting relatives ($n = 11$); (iii) non-carrier relatives ($n = 9$); (iv) external control subjects ($n = 11$). Patients affected by DYT1 dystonia and their unaffected relatives, including 6 first-degree relatives (all DYT1 carriers), 7 second-degree relatives (4 DYT1 carriers, 3 non-carriers), 4 third-degree relatives (1 DYT1 carrier, 3 non-carriers) and 3 fifth-degree relatives (all non-carriers) belonged to three Italian families. One family originated from a small countryside area close to Bergamo (Northern Italy). Further information about DYT1 atypical phenotypes in this family (Patients 1–4 in Table 1) can be found in our recent description (Gambarin *et al.*, 2006). The other two families (one of which has been described in Bentivoglio *et al.*, 2002) originated from Sardinia (one from the city Cagliari and one from a village nearby, Villaputzu). Inclusion criteria for all groups were the absence of other neurological diseases and normal sight, or corrected to normal.

Table 1 Patients' demographic and clinical information

Patient/gender	Age/education (years)	Age at onset (years)	Site of onset	Current distribution of dystonia	Severity score[a]	Therapy[b]
1/F	25/11	10	L arm	Segmental	17	No
2/M	50/5	43	Neck	Generalized	22	BTX
3/M	28/8	22	R arm	Focal	2	No
4/F	69/8	43	Neck	Generalized	38.5	BTX
5/M	37/8	8	R leg	Generalized	65.5[c]	DBS
6/F	68/11	8	R leg	Segmental	38.5[c]	DBS
7/F	28/13	20	L arm	Focal	2[c]	DBS
8/F	48/13	39	L arm	Focal	6	No
9/M	27/13	8	R leg	Generalized	124	DBS

[a]Burke–Fahn–Marsden movement and disability scale; [b]BTX: botulinum toxin; DBS: deep brain stimulator in the globus pallidus pars interna; [c]Evaluation with stimulators on.

DYT1 manifesting patients

This group was made up of nine patients (five women and four men) with clinical diagnosis of definite PTD and presence of the GAG deletion in the DYT1 gene. Demographic and clinical information about this group is provided in Table 1. Age ranged from 25 to 69 years (mean: 42.2 ± 17.5 years), education level from 5 to 13 years (mean: 10.0 ± 2.9 years) and duration of disease from 6 to 59 years (mean: 19.9 ± 17.2 years). Severity of motor impairment was evaluated by using the Burke–Fahn–Marsden movement and disability scale (Burke et al., 1985). Three patients (Patients 1, 3 and 8) were untreated; two patients (Patients 2 and 4) had received repeated treatments with botulinum toxin until 6 months before the study, without significant improvement of symptoms, and four patients (Patients 5–7 and 9) had a deep brain stimulator bilaterally located in the globus pallidus pars interna. These patients performed the tasks keeping the stimulators on for two main reasons. First, three of them (Patients 5, 6 and 9) had high dystonia severity scores even with stimulators on (see Table 1) and, in principle, turning the stimulator off in these patients would have been unethical. Second, although mild improvements in dystonia are observed within a few hours after switching on the stimulators, it is widely held that it may take months for the full effect of pallidal stimulation to develop, and therefore we reasoned that switching off the stimulators a few hours before the test was unlikely to affect patients' performance (Kumar et al., 1999; Coubes et al., 2000; Volkmann and Benecke, 2002).

DYT1 non-manifesting relatives

Among relatives of DYT1 dystonic patients, 11 healthy carriers of the DYT1 mutation (6 women and 5 men) without clinical manifestation of dystonia were recruited. Age ranged from 17 to 79 years (mean: 49.9 ± 21.5 years) and education level ranged from 5 to 13 years (mean: 9.0 ± 3.5 years).

Non-carrier relatives

From the relatives of DYT1 dystonic patients, 9 healthy individuals (4 women and 5 men) who did not carry the DYT1 mutation were recruited so as to rule out any effect due to non-specific impairment of sensory skills in those families. Age ranged from 19 to 85 years (mean: 36.1 ± 20.8 years) and education level ranged from 5 to 13 years (mean: 9.9 ± 3.1 years).

External control subjects

Also, 11 healthy control subjects (7 women and 4 men) were recruited. Age ranged from 27 to 61 years (mean: 36.6 ± 10.9 years) and education level ranged from 5 to 18 years (mean: 12.3 ± 5.7 years). These participants did not belong to the DYT1 families and did not carry the GAG deletion.

Mean ages and education levels of these four groups were analysed by means of one-way ANOVA. All subjects gave their written informed consent prior to participation in the study. The procedure was approved by the Institutional Ethics Committee, and the study was carried out in accordance with the ethical standards of the 1964 Declaration of Helsinki.

Stimuli and procedure

Tactile stimuli in a pair consisted of square wave electrical pulses delivered by means of a constant current stimulator (STM 140, HTL, Udine, Italy) through surface skin electrodes (1 mm diameter) applied to the index and middle fingers of the right or the left hand. The anode was located 1.5 cm distally from the cathode. For each subject, the intensity of tactile stimulation was determined by delivering a series of stimuli with increasing intensity (from 2 mA in steps of 1 mA). The minimal intensity at which electric stimuli were perceived in 10 out of 10 stimuli was used in the experimental test. Care was taken that stimuli did not induce pain or discomfort. Mean intensity of stimulation used in the four groups was analysed using one-way ANOVA.

Visual stimuli were delivered through light emitting diodes (LEDs) positioned on a black table (51×37 cm^2) at 57 cm from the subject's head and 7° left or right from a central fixation point. The luminance of each LED was 140 cd/m^2 and the background luminance was about 15 cd/m^2. Both visual and tactile stimuli lasted 5 ms. Subjects' hands were positioned near the LEDs. Subjects were asked to look at the fixation point throughout each trial. The maintenance of fixation was controlled directly by the experimenter. In patients affected by neck muscle contractions, their head was kept straight by an examiner during delivery of stimuli. Trials in which participants did not maintain fixation (∼2%) were discarded. A schematic representation of the different stimulation conditions is provided in Fig. 1.

Subjects were tested in one experimental session lasting ∼90–120 min. The experimental test was delivered in six combinations of stimulation: two visual (left and right), two tactile (left and right) and two cross-modal (vision–touch left and vision–touch right) (see Fig. 1). The order of presentation of the six combinations of stimuli was counterbalanced across subjects. Each combination of stimuli was performed in four separate blocks. In the first trial of each block, pairs of simultaneous stimuli [inter-stimulus interval (ISI) = 0 ms] were delivered. In subsequent trials, ISIs were progressively increased in steps of 10 ms. We considered as TDT the first out of three consecutive ISIs at which subjects recognized the stimuli as asynchronous. Subjects were also asked to judge which stimulus preceded (or followed) the other. Temporal order judgement (TOJ) corresponds to the first of three consecutive ISIs at which subjects not only recognized the stimuli as separated in time, but also reported correctly which stimulus in the pair preceded (or followed) the other. While TDT provides a measure of one's capability to detect synchrony/asynchrony, TOJ is likely to tap higher-order abilities such as language and memory.

Statistical analysis

For each performance index (TDT and TOJ), averages of 4 values, one for each block, were entered in the data analysis. We analysed TDT and TOJ by means of two different analyses of variance (ANOVA) with repeated measures. Each ANOVA had one between-subjects factor, 'group' (DYT1 manifesting, DYT1 non-manifesting, non-carrier relatives, external control subjects), and two within-subjects factors: 'combination of stimuli' (visual, tactile and visuo-tactile) and 'side of stimulation' (right and left). Post hoc comparisons were carried out by using t-tests with Bonferroni correction.

The Spearman correlation coefficient was used for assessing the possible relationships between the dystonia severity score (Burke et al., 1985) and performances in temporal discrimination (TDT and TOJ) of visual, tactile and visuo-tactile stimuli in DYT1 manifesting patients.

To further assess the specific role of phenotype (i.e. presence of motor symptoms) in determining performance deficits, we
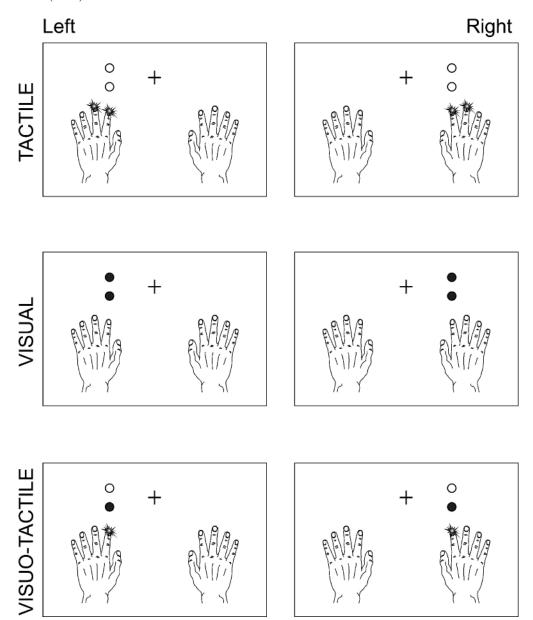
Fig. 1 Schematic representation of the experimental conditions. In the visual combination two flashes (grey spots in the picture) were delivered through LEDs positioned to the right or to the left visual field. In the tactile combinations, two electrical stimuli were delivered on the volar surface of the distal phalanx of the index and middle fingers of both hands. In the visuo-tactile combination, one electric stimulus and one flash (grey spot) were delivered within the same personal and peripersonal hemispace (either the right or the left).

calculated the mean difference in TDT and TOJ between manifesting and non-manifesting *DYT1* mutation carriers. Since these two groups have the same genotype, but they differ in the presence of the phenotypic expression, any impairments in the sensory performance may be linked to the presence of motor symptoms. In a similar vein, to investigate for any specific effects related to the genotype (presence of the genetic mutation), we calculated the mean difference between DYT1 non-manifesting carriers and non-carrier relatives. These groups have in common the absence of motor symptoms, but they differ for the presence of the DYT1 mutation, therefore any difference might be ascribed to the presence of such mutation. Group differences ('DYT1 manifesting minus DYT1 non-manifesting' and 'DYT1

non-manifesting minus non-carrier relatives') were first computed for each stimulus combination and each side of stimulation, for a total of six values (visual left, visual right, tactile left, tactile right, visuo-tactile left, visuo-tactile right). The two group differences in the six cells were then contrasted by means of *t*-tests for independent samples.

Results
Preliminary *t*-tests for independent samples showed that TDT and TOJ values in visual, tactile and visual-tactile combinations were comparable in patients who had
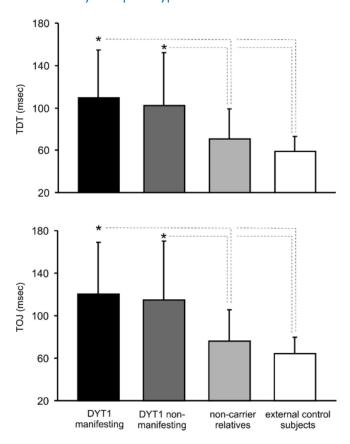
Fig. 2 Mean values and standard deviations of TDT and TOJ in DYT1 manifesting patients, DYT1 non-manifesting relatives, non-carrier relatives and external control subjects, by plotting all combinations of stimuli together (visual, tactile and visuo-tactile).
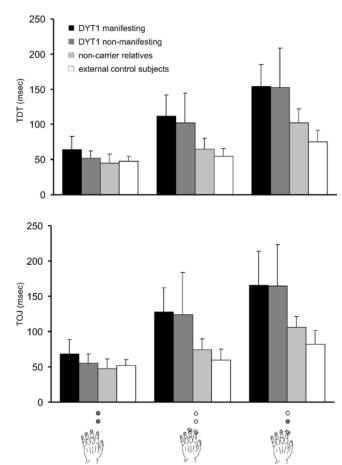

Fig. 3 Mean values and standard deviations of TDT and TOJ in DYT1 manifesting patients, DYT1 non-manifesting relatives, non-carrier relatives and external control subjects in the three combinations of stimuli separately.

implantation for deep brain stimulation ($n = 4$) and patients without implantation ($n = 5$; for all comparisons: $P > 0.408$).

Mean age and education levels of the four groups were not significantly different (ANOVA, age: $P = 0.278$; education: $P = 0.292$). Mean intensity of stimulation used in DYT1 manifesting patients (10 mA, SD 8.0), in DYT1 non-manifesting relatives (9 mA, SD 14.0), in non-carrier relatives (7 mA, SD 4.0) and in control subjects (6 mA, SD 7.0) was also comparable (ANOVA, $P = 0.785$).

Figure 2 shows TDTs and TOJs of DYT1 manifesting patients, DYT1 non-manifesting relatives, non-carrier relatives and external control subjects.

The analysis of variance showed a significant effect of the factor 'group' [TDT: $F(3,36) = 12.6$, $P < 0.001$; TOJ: $F(3,36) = 11.3$, $P < 0.001$]. This effect was due to a better performance of non-carrier relatives (TDT: 70.7 ms; TOJ: 76.1 ms) and external control subjects (TDT: 59.0 ms; TOJ: 64.1 ms) compared with DYT1 carriers, both manifesting (TDT: 109.7 ms; TOJ: 120.2 ms) and non-manifesting (TDT: 101.9 ms; TOJ: 114.5 ms). No difference was observed within the first two groups and within the last two groups (*see* Fig. 2).

The factor 'combination of stimuli' was also significant [TDT: $F(2,72) = 113.1$, $P < 0.001$; TOJ: $F(2,76) = 94.2$,

$P < 0.001$], insofar as visuo-tactile combinations (TDT: 120.6 ms; TOJ: 129.4 ms) were more difficult than tactile (TDT: 83.0 ms; TOJ: 96.2 ms) and visual (TDT: 52.2 ms; TOJ: 55.6 ms) combinations. Moreover, temporal thresholds in the tactile combinations were significantly higher than in visual combinations. The interaction 'group' × 'combination of stimuli' [TDT: $F(6,72) = 7.3$, $P < 0.001$; TOJ: $F(6,72) = 6.7$, $P < 0.001$] was also significant (Fig. 3).

Post hoc comparisons showed that both groups of DYT1 carriers (manifesting and non-manifesting) were significantly more impaired than non-carrier relatives and control subjects in visuo-tactile and tactile tasks (for all comparisons: TDT: $P < 0.039$; TOJ: $P < 0.032$). Moreover, manifesting DYT1 dystonic patients had abnormal performance also in the visual combinations compared to non-carrier relatives (TDT: $P = 0.023$; TOJ: $P = 0.025$) and external control subjects (TDT: $P = 0.047$).

Mean values and standard deviations of the TDT and TOJ measurements in each combination of stimuli are provided in Supplementary tables in *Brain* Online.

No other effects or interactions were significant. In particular, the insignificance of the triple interaction
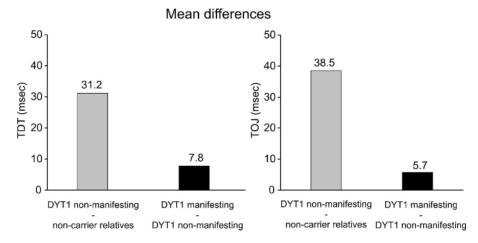
Mean differences

Fig. 4 Mean differences of TDT and TOJ between DYT1 non-manifesting relatives and non-carrier relatives (grey bars) and between DYT1 manifesting patients and DYT1 non-manifesting relatives (black bars).

'group' × 'combination of stimuli' × 'side' carries the important implication that performance was comparable on the left and right side not only in controls but also in DYT1 carriers.

In order to define a threshold value in our task discriminating between *DYT1* mutation carriers and control subjects, we calculated the upper limit of normal TDT. This value was determined as the mean TDT of the external control group plus 2.5 SD. Any TDT greater than this value could be considered abnormal. The upper limit of external control subjects has been calculated for the tactile and visuo-tactile combinations of stimuli, since only these two conditions were significantly different between non-manifesting DYT1 carriers and controls. These values were 83.3 ms in the tactile condition and 116.7 ms in the visuo-tactile condition. In the tactile condition, 7 out of 11 non-manifesting DYT1 carrier relatives had abnormal TDT, while only 1 of 9 non-carrier relatives was above the limit. In the visuo-tactile condition, 8 of 11 non-manifesting DYT1 carriers and 2 of 9 non-carrier relatives were outside the normal limit.

No correlation between severity of disease and performance in the temporal discrimination tasks was found (TDT: $P > 0.349$; TOJ: $P > 0.381$).

Figure 4 shows a significantly increased 'DYT1 non-manifesting—non-carrier relatives' difference in respect to the 'DYT1 manifesting–DYT1 non-manifesting' difference [TDT: $t(10) = 2.5$, $P = 0.030$; TOJ: $t(10) = 2.9$, $P = 0.015$]. This suggests that the presence of the *DYT1* mutation is sufficient to determine temporal discrimination deficits. Moreover, this finding allows us to rule out any effect due to a general, unspecific impairment of those families in performing temporal discrimination tasks.

Discussion

Our study highlights, for the first time, a tight link between the presence of the DYT1 GAG deletion and the ability to perceive visual, tactile or visuo-tactile stimuli as temporally separated. More specifically, higher TDTs of tactile and visuo-tactile stimuli have been found in DYT1 carriers, both manifesting and non-manifesting, when compared to non-carrier relatives and to external control subjects. Additional visual impairment was found in DYT1 manifesting patients but not in non-manifesting carriers, suggesting that sensory deficits are more extended in the former than in the latter group. The visual temporal discrimination deficits found in our patients may be related to the fact that most of them had dystonia extended to more than one body part (two segmental and four generalized). As previously suggested, deficits of temporal discrimination seem to vary along both quantitative (intensive) and qualitative (extensive) dimensions, depending on the distribution of symptoms (Tinazzi et al., 2004). More specifically, temporal deficits for tactile and visuo-tactile stimuli have been observed in both focal (Fiorio et al., 2003; Tinazzi et al., 2004) and generalized dystonia (Aglioti et al., 2003), while temporal discrimination of visual stimuli was affected only in the latter form. These findings have been explained by assuming that while local somaesthetic factors may be involved in focal dystonia, a more general basal ganglia-related timing function may be altered in generalized dystonia (Tinazzi et al., 2004).

Despite the fact that the patients' group was small and inhomogeneous, it was statistically more impaired in the task than non-carrier relatives and external control subjects, thus suggesting that the number of patients was enough to reach a significant difference level.

The present results on temporal discrimination seem to contrast with previous studies on spatial discrimination (Molloy et al., 2003; O'Dwyer et al., 2005). Deficits of spatial discrimination of cutaneous stimuli were found in focal dystonias, including focal-hand dystonia, blepharospasm and cervical dystonia, but not in DYT1 generalized dystonia (Molloy et al., 2003). This raises the possibility that sensory deficits in spatial and temporal discrimination tasks are differently expressed in different forms of dystonia.

However, since the number of studies on the sensory abilities of DYT1 patients is still limited, and the number of patients studied in spatial and temporal tasks is low, we cannot exclude that patients might be deficient in both tasks. To the best of our knowledge, temporal discrimination of tactile stimuli is a complex task that, as indicated by functional imaging studies in healthy subjects, as well as by studies in patients with lesions or chronic neurological disease, impinges upon several cortical and subcortical areas, such as pre-supplementary motor area, anterior cingulate cortex and basal ganglia, in addition to the primary sensory areas (Lacruz et al., 1991; Artieda et al., 1992; Harrington et al., 1998b; Pastor et al., 2004, 2006). It is worth noting that this pattern of activation corresponds roughly to brain areas where metabolic changes in DYT1 gene carriers have been observed (Eidelberg et al., 1998; Trošt et al., 2002). Spatial discrimination, instead, mainly requires activation in the frontal eye field, ventral premotor cortex, postcentral sulcus, anterior intraparietal sulcus, parietooccipital cortex and angular gyrus (Zhang et al., 2005). It would be interesting to investigate whether other genetic mutations related to dystonia might be responsible for subclinical alterations in these brain areas, thus causing merely spatial discrimination deficits.

The temporal tactile and visuo-tactile deficits found in the present study are clearly associated to the presence of the DYT1 gene mutation and not to the phenotypic expression of dystonia. Indeed, in spite of the high variation of the dystonic symptoms (generalized, focal and segmental), all the patients of this study were clearly impaired in performing temporal discrimination tasks. Moreover, difference in performance between DYT1 non-manifesting carriers and non-carrier relatives was higher than the difference between DYT1 manifesting patients and DYT1 non-manifesting carriers. This suggests that the temporal discrimination deficits in tactile and visuo-tactile tasks reflect a genuine contribution of the genetic mutation. No difference in performance has been found between family members who did not carry the genetic mutation and external control subjects, suggesting that the families we examined have normal sensory skills, whenever the gene is not mutated.

Previous studies on temporal processing of tactile and/or visuo-tactile inputs in PTD highlighted the importance of the sensory systems in the pathophysiology of dystonia (Tinazzi et al., 1999, 2002, 2004; Bara-Jimenez et al., 2000; Sanger et al., 2001; Aglioti et al., 2003; Fiorio et al., 2003). Most of these studies have emphasized the specific role of the peripheral inputs involved in the motor control of the dystonic body part.

However, by applying the temporal discrimination paradigm to focal-hand dystonia patients, we have reported that, despite the unilateral clinical manifestation of motor symptoms (right hand), abnormal thresholds for tactile and visuo-tactile stimuli were present in the affected as well as in the unaffected side (Fiorio et al., 2003). Therefore,

abnormalities in the somatosensory domain may be present independently from the localization of motor symptoms. The sensory abnormalities in the unaffected hand parallel the findings of a magneto-encephalography study in unilateral task-specific dystonia in which a clear disorganization of the somatic representation of the dystonic and non-dystonic hand was found (Meunier et al., 2001). The two studies (Meunier et al., 2001; Fiorio et al., 2003) converged to indicate that sensory abnormalities of the non-dystonic hand can be considered as endophenotypic traits of dystonia. Similarly, a bilateral increase in grey matter volume was found in the hand representation area of primary somatosensory and motor cortices of patients with unilateral focal-hand dystonia (Garraux et al., 2004).

Thus, some alterations might occur before the appearance of dystonic signs and might be regarded as a subclinical phenotype in DYT1 mutation carriers independent of the manifestation of motor symptoms. The factors causing these subclinical abnormalities are still unknown. However, recent studies hint at the possible role of the DYT1 mutation in inducing a subclinical 'susceptibility substrate' to develop dystonia (Eidelberg et al., 1998; Edwards et al., 2003). Indeed, PET studies have revealed that regardless of clinical manifestation of dystonia, DYT1 carriers show abnormal pattern of glucose utilization, characterized by hypermetabolism of some cortical and subcortical areas, such as the supplementary motor area, the basal ganglia and the cerebellum (Eidelberg et al., 1998; Trošt et al., 2002). These findings allowed Eidelberg et al. (1998) to firstly develop the concept of DYT1 endophenotype. Evidence for the role of the DYT1 mutation in determining subclinical alterations also comes from an electrophysiological study in which abnormalities of motor cortical inhibition (intracortical inhibition and silent period) have been found in both manifesting and non-manifesting DYT1 carriers (Edwards et al., 2003).

The present study significantly confirms previous knowledge on sensory alterations in dystonia (Meunier at al., 2001; Fiorio et al., 2003) and expands the DYT1 endophenotype concept proposed by Eidelberg et al. (1998), by showing that temporal discrimination deficits are not strictly linked to the presence of dystonic symptoms. An entirely novel finding is that abnormal TDTs and TOJs of tactile and visuo-tactile inputs were found in the family members carriers of the DYT1 mutation, in the complete absence of motor manifestation of disease.

The general meaning of the sensory abnormality associated to the DYT1 mutation is, however, unclear. On the one hand, temporal discrimination deficits may be a mere epiphenomenon of the primary disorder underlying DYT1 dystonia that can be explained by dysfunctions of a neural network involving basal ganglia. This set of structures is implicated not only in sensory-motor control but also in higher-order functions (Bares and Rektor, 2001; Jahanshahi et al., 2002; Koechlin et al., 2002), such as motor and perceptual timing operations (Ivry, 1996; Harrington et al.,

1998*a*; Rao *et al.*, 2001), and integration of sensory signals coming from different modalities (Graziano and Gross, 1993; Graziano *et al.*, 1994). Interestingly, functional studies have shown that the protein product of the *TOR1A/DYT1* gene, *TorsinA*, is highly expressed in specific sets of neurons such as dopaminergic neurons of the striatum (Konakova *et al.*, 2001), and, when mutated, tends to form perinuclear membrane whorls which can disrupt or divert trafficking of membrane-bound proteins, such as the dopamine transporters. This process interferes with generation and maintenance of synaptic connections making neurons more susceptible to stress (Shashidharan *et al.*, 2000; Breakefield *et al.*, 2001; Torres *et al.*, 2004; Naismith *et al.*, 2004). Moreover, the disruptive effect of mutant *TorsinA* appears to be restricted to neuronal cells, suggesting that selected groups of neurons may have a unique requirement for *TorsinA* normal function (Goodchild *et al.*, 2005). However, it must be noted that these experiments have been performed in cellular models and thus their relevance to function and disease remains to be determined.

On the other hand, temporal discrimination deficits may arise from disorders affecting sensory systems primarily involved in the pathophysiology of *DYT1* dystonia. Given the crucial role of sensation for action, disordered sensations can lead to disordered movements. A pre-existing sensory alteration in *DYT1* carriers may allow other factors to trigger the motor symptoms. In principle, factors overloading a vulnerable sensory system might enhance the risk of developing dystonia in predisposed subjects. Although peripheral injury seems to play an important role in triggering topographically related dystonic symptoms in late-onset dystonia, there is no proven evidence that the same is happening in DYT1 dystonia.

The detection of raised TDT in late-onset dystonia is intriguing. The suggestion that sensory deficits can be useful biological markers of a genetic status in adult-onset focal dystonia is supported by the finding of somatosensory spatial discrimination abnormalities not only in adult-onset focal dystonia patients but also in some of their relatives without dystonia (O'Dwyer *et al.*, 2005), who could be non-penetrant carriers of a mutation in a still unknown gene.

In the present study, not all relatives carrying the *DYT1* mutation had abnormal TDT values. Four of eleven non-manifesting DYT1 carriers fell within the normal range in the tactile condition and three of them had normal TDT in the visuo-tactile condition. The proportion of false-negatives was ∼36.4% for the tactile condition and 27.3% for the visuo-tactile condition. Vice versa, eight of nine non-carrier relatives were in the normal limit range in the tactile condition and seven of them were below the limit also in the visuo-tactile condition. The proportion of false positives was therefore 11.1% in the tactile condition and 22.2% in the visuo-tactile condition. It should be noticed, however, that the number of subjects in our study is low and does not allow us to consider these thresholds as absolute and generalized values. Future studies on larger samples of control subjects and DYT1 carriers and non-carriers are needed to better define a precise TDT value discriminating between groups.

In conclusion, by using a simple psychophysical task we have been able to highlight a clear association between the *DYT1* genetic mutation and sensory deficits. Therefore, dystonic motor symptoms might not be the exclusive phenotypic expression of the *DYT1* mutation, being sensory deficits expressed as well. Even more, an important implication of the present study is that, while the mutated *DYT1* gene has a 30–40% penetrance in inducing the 'dystonic phenotype', it seems to have a higher penetrance in determining sensory deficits. Although, at present the sensitivity and specificity of our test are still quite low, we reason that, in the future, a screening based on our psychophysical paradigm might help to disclose the 'sensory phenotype' in family members of dystonic patients and might also be considered as a possible marker of mutation carriage, easing the identification of novel dystonia genes through familial genetic studies.

Supplementary material
Supplementary data are available at *Brain* Online.

Acknowledgements

References
Abbruzzese G, Marchese R, Buccolieri A, Gasparetto B, Trompetto C. Abnormalities of sensorimotor integration in focal dystonia: a transcranial magnetic stimulation study. Brain 2001; 124: 537–45.

Aglioti SM, Fiorio M, Forster B, Tinazzi M. Temporal discrimination of cross-modal and unimodal stimuli in generalized dystonia. Neurology 2003; 60: 782–5.

Artieda J, Pastor MA, Lacruz F, Obeso JA. Temporal discrimination is abnormal in Parkinson's disease. Brain 1992; 115: 199–210.

Bara-Jimenez W, Shelton P, Sanger TD, Hallett M. Sensory discrimination capabilities in patients with focal hand dystonia. Ann Neurol 2000; 47: 377–80.

Bares M, Rektor I. Basal ganglia involvement in sensory and cognitive processing. A depth electrode CNV study in human subjects. Clin Neurophysiol 2001; 112: 2022–30.

Bentivoglio AR, Loi M, Valente EM, Ialongo T, Tonali P, Albanese A. Phenotypic variability of DYT1-PTD: does the clinical spectrum include psychogenic dystonia? Mov Disord 2002; 17: 1058–63.

Breakefield XO, Kamm C, Hanson PI. TorsinA: movement at many levels. Neuron 2001; 31: 9–12.

Bressman SB. Dystonia. Curr Opin Neurol 1998; 11: 363–72.

Burke RE, Fahn S, Marsden CD, Bressman SB, Moskowitz C, Friedman J. Validity and reliability of a rating scale for the primary torsion dystonias. Neurology 1985; 35: 73–7.

Coubes P, Roubertie A, Vayssiere N, Hemm S, Echenne B. Treatment of DYT1-generalised dystonia by stimulation of the internal globus pallidus. Lancet 2000; 355: 2220–1.

Edwards MJ, Huang YZ, Wood NW, Rothwell JC, Bhatia KP. Different patterns of electrophysiological deficits in manifesting and non-manifesting carriers of the DYT1 gene mutation. Brain 2003; 126: 2074–80.

Eidelberg D, Moeller JR, Antonini A, Kazumata K, Nakamura T, Dhawan V, et al. Functional brain networks in DYT1 dystonia. Ann Neurol 1998; 44: 303–12.

Fahn S, Bressman SB, Marsden CD. Classification of dystonia. In: Fahn S, Marsden CD, DeLong MR, editors. Advances in neurology. Vol. 78. Philadelphia: Lippincott-Raven; 1998. p. 1–10.

Fiorio M, Tinazzi M, Bertolasi L, Aglioti SM. Temporal processing of visuotactile and tactile stimuli in writer's cramp. Ann Neurol 2003; 53: 630–5.

Gambarin M, Valente EM, Liberini P, Barrano G, Ionizzato A, Padovani A, et al. Atypical phenotypes and clinical variability in a large Italian family with DYT1-primary torsion dystonia. Mov Disord. 2006 Jul 27; [Epub ahead of print].

Garraux G, Bauer A, Hanakawa T, Wu T, Kansaku K, Hallett M. Changes in brain anatomy in focal hand dystonia. Ann Neurol 2004; 55: 736–9.

Goodchild RE, Kim CE, Dauer WT. Loss of the dystonia-associated protein torsinA selectively disrupts the neuronal nuclear envelope. Neuron 2005; 48: 923–32.

Graziano MS, Gross CG. A bimodal map of space: somatosensory receptive fields in the macaque putamen with corresponding visual receptive fields. Exp Brain Res 1993; 97: 96–109.

Graziano MS, Yap GS, Gross CG. Coding of visual space by premotor neurons. Science 1994; 266: 1054–7.

Hallett M. Is dystonia a sensory disorder? Ann Neurol 1995; 38: 139–40.

Harrington DL, Haaland KY, Hermanowicz N. Temporal processing in the basal ganglia. Neuropsychology 1998a; 12: 3–12.

Harrington DL, Haaland KY, Knight RT. Cortical networks underlying mechanisms of time perception. J Neurosci 1998b; 18: 1085–95.

Ivry RB. The representation of temporal information in perception and motor control. Curr Opin Neurobiol 1996; 6: 851–7.

Jahanshahi M, Rowe J, Saleem T, Brown RG, Limousin-Dowsey P, Rothwell JC, et al. Striatal contribution to cognition: working memory and executive function in Parkinson's disease before and after unilateral posteroventral pallidotomy. J Cogn Neurosci 2002; 14: 298–310.

Koechlin E, Danek A, Burnod Y, Grafman J. Medial prefrontal and subcortical mechanisms underlying the acquisition of motor and cognitive action sequences in humans. Neuron 2002; 35: 371–81.

Konakova M, Huynh DP, Yong W, Pulst SM. Cellular distribution of torsin A and torsin B in normal human brain. Arch Neurol 2001; 58: 921–7.

Kumar R, Dagher A, Hutchison WD, Lang AE, Lozano AM. Globus pallidus deep brain stimulation for generalized dystonia: clinical and PET investigation. Neurology 1999; 53: 871–4.

Lacruz F, Artieda J, Pastor MA, Obeso JA. The anatomical basis of somaesthetic temporal discrimination in humans. J Neurol Neurosurg Psychiatry 1991; 54: 1077–81.

Meunier S, Garnero L, Ducorps A, Mazieres L, Lehericy S, du Montcel ST, et al. Human brain mapping in dystonia reveals both endophenotypic traits and adaptive reorganization. Ann Neurol 2001; 50: 521–7.

Molloy FM, Carr TD, Zeuner KE, Dambrosia JM, Hallett M. Abnormalities of spatial discrimination in focal and generalized dystonia. Brain 2003; 126: 2175–82.

Naismith TV, Heuser JE, Breakefield XO, Hanson PI. TorsinA in the nuclear envelope. Proc Natl Acad Sci USA 2004; 101: 7612–7.

Nemeth AH. The genetics of primary dystonias and related disorders. Brain 2002; 125: 695–721.

O'Dwyer JP, O'Riordan S, Saunders-Pullman R, Bressman SB, Molloy F, Lynch T, et al. Sensory abnormalities in unaffected relatives in familial adult-onset dystonia. Neurology 2005; 65: 938–40.

Ozelius LJ, Hewett JW, Page CE, Bressman SB, Kramer PL, Shalish C, et al. The early-onset torsion dystonia gene (DYT1) encodes an ATP-binding protein. Nat Genet 1997; 17: 40–8.

Pastor MA, Day BL, Macaluso E, Friston KJ, Frackowiak RSJ. The functional neuroanatomy of temporal discrimination. J Neurosci 2004; 24: 2585–91.

Pastor MA, Macaluso E, Day BL, Frackowiak RS. The neural basis of temporal auditory discrimination. Neuroimage 2006; 30: 512–20.

Rao SM, Mayer AR, Harrington DL. The evolution of brain activation during temporal processing. Nat Neurosci 2001; 4: 317–23.

Sanger TD, Tarsy D, Pascual-Leone A. Abnormalities of spatial and temporal sensory discrimination in writer's cramp. Mov Disord 2001; 16: 94–9.

Saunders-Pullman R, Shriberg J, Shanker V, Bressman SB. Penetrance and expression of dystonia genes. Adv Neurol 2004; 94: 121–5.

Shashidharan P, Kramer BC, Walker RH, Olanow CW, Brin MF. Immunohistochemical localization and distribution of torsinA in normal human and rat brain. Brain Res 2000; 853: 197–206.

Tinazzi M, Frasson E, Bertolasi L, Fiaschi A, Aglioti S. Temporal discrimination of somesthetic stimuli is impaired in dystonic patients. Neuroreport 1999; 10: 1547–50.

Tinazzi M, Fiaschi A, Frasson E, Fiorio M, Cortese F, Aglioti SM. Deficits of temporal discrimination in dystonia are indipendent from the spatial distance between the loci of tactile stimulation. Mov Disord 2002; 17: 333–8.

Tinazzi M, Rosso T, Fiaschi A. Role of the somatosensory system in primary dystonia. Mov Disord 2003; 18: 605–22.

Tinazzi M, Fiorio M, Bertolasi L, Aglioti SM. Timing of tactile and visuo-tactile events is impaired in patients with cervical dystonia. J Neurol 2004; 251: 85–90.

Torres GE, Sweeney AL, Beaulieu JM, Shashidharan P, Caron MG. Effect of torsinA on membrane proteins reveals a loss of function and a dominant-negative phenotype of the dystonia-associated DeltaE-torsinA mutant. Proc Natl Acad Sci USA 2004; 101: 15650–5.

Trošt M, Carbon M, Edwards C, Ma Y, Raymond D, Mentis MJ, et al. Primary dystonia: is abnormal functional brain architecture linked to genotype? Ann Neurol 2002; 52: 853–6.

Valente EM, Warner TT, Jarman PR, Mathen D, Fletcher NA, Marsden CD, et al. The role of DYT1 in primary torsion dystonia in Europe. Brain 1998; 121: 2335–9.

Volkmann J, Benecke R. Deep brain stimulation for dystonia: patient selection and evaluation. Mov Disord 2002; 17: S112–5.

Zhang M, Mariola E, Stilla R, Stoesz M, Mao H, Hu X, et al. Tactile discrimination of grating orientation: fMRI activation patterns. Hum Brain Mapp 2005; 25: 370–7.