Abnormal blink reflex recovery cycle in manifesting and non-manifesting carriers of the DYT1 gene mutation

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Running head: Abnormal blink reflex recovery in DYT1

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Abstract

Objective: To evaluate the brainstem function in DYT1 carriers manifesting clinical dystonia (MDYT1) and those without clinical symptoms (NMDYT1).

Background: Motor cortical inhibition and plasticity were found abnormal in MDYT1, while those were less abnormal in NMDYT1. On the other hand, the spinal reciprocal inhibition was abnormal in MDYT1, but normal in NMDYT1. Moreover, protein accumulation and perinuclear inclusion bodies was found in the brainstem, but not other brain areas, in DYT1 patients. Therefore we designed this study to investigate the brainstem physiology using the blink reflex recovery cycle test in NDYT1 and NMDYT1.

Methods: We recruited eight MDYT1, five NMDYT1 and nine age-matched healthy controls. The blink reflex recovery cycle (BR) was assessed with paired stimuli that evoked the blink reflex in a random order at interstimulus intervals of 250, 500 and 1000ms.

Results: A two-way ANOVA showed a significant difference betweenMDYT1, NMDYT1 and the healthy control (p=0.004). Post hoc analysis showed this was due to a significantly less inhibition of R2 in MDYT1 and NMDYT1 as compared to controls (2-way ANOVA: p=0.003, p=0.021, respectively). There was no difference between MDYT1 and NMDYT1 (p=0.224).

Conclusions: The tested brainstem circuits were equally involved in MDYT1 and NMDYT1. The finding is compatible with the pathological findings in DYT1 carriers. Together with previous findings in the motor cortex and spinal cord, brainstem may lies closer to the pathogenesis of dystonia than the motor cortex in DYT1 gene carriers.

Key words: DYT1, blink reflex, pathophysiology, dystonia

Introduction

Dystonia is a kind of hyperkinetic movement disorder with clinical feature of abnormal sustained limbs or trunk twisting posture. The neurophysiology studies have revealed dysfunction in basal ganglion-sensorimotor network [1-3], dysfunction in cerebellothalamocortical pathway [4,5], reduced cortical inhibition with increased cortical plasticity [6-8], abnormal premotor-motor connectivity [9,10] and decreased brain stem inhibition [1,3,11,12] and reduced spinal cord reciprocal inhibition [13,14]. , Recent findings suggested that dystonia could be a brain network disorder, and the basal ganglion may not the primary source to develop the entire dysfunction network of dystonia [15,16]. Hence, the exact pathogenesis of dystonia has been unclear so far.

In primary dystonia, DYT1 related dystonia is the most common cause of young onset primary general dystonia [17]. DYT1 dystonia is a familial early-onset dystonia due to a single GAG deletion in the DYT1 gene and produce the abnormal TorsinA protein with a single glutamate residue deletion in the C-terminus [18]. Although DYT1 related dystonia is an autosomal dominant disorder, only 30-40 % of penetrating rate that make some gene carriers eventually develop dystonia. The others may not manifest any limbs or truncal

twisting symptoms [11]. Hence, it would be helpful for understanding the pathogenesis of dystonia by clarify the pathophysiology of DYT1 gene mutation carriers with clinical manifesting dystonia (MDTY1) and without dystonia (NMDYT1). A previous study discovered that the motor cortical inhibition was reduced in both MDTY1 and NMDYT1 subjects, although the reduction in short interval intracortical inhibition (SICI) was minor in NMDYT1 than in MDYT1 subjects [8]. Besides, motor plasticity in response to theta burst stimulation from of rTMS was enhanced in MDYT1, but reduced in NMDYT1 subjects [7]. In contrast, the spinal reciprocal inhibition was reduced in MDYT1, but normal in NMDYT1 [8]. The results indicate that motor cortical plasticity and inhibitory circuits are abnormal in both MDYT1 and NMDYT1 subjects, while the spinal cord inhibition is abnormal in MDYT1 only.

A pathology study of MDYT1 revealed that protein accumulation and perinuclear inclusion bodies presented only in brainstem, not basal ganglion or cortex [20]. In addition, a recently study of the eye blink physiology also showed enhanced blink reflex recovery curve in DYT1 dystonia patients [12]. Therefore, it would be valuable to compare and contrast the brainstem physiology of MDYT1 and NMDYT1. For this purpose, we arranged this study

to evaluate the blink reflex recovery cycle in MDYT1 and NMDYT1.

Method

Subjects

We recruited eight DYT1 gene carriers (4 men and 4 women with average age 46 ± 13.76) manifesting dystonia symptoms (MDYT1) and five carriers (4 men and 1 woman with average 43.6 ± 15.43) without manifesting dystonia symptoms (NMDYT1) from the movement disorder clinics at the National Hospital for Neurology and Neurosurgery in London, UK and at the Chang Gung Memorial Hospital at Linkou, Taiwan. Nine age-matched healthy subjects (6 men, 3 women, average age 46 ± 7.05) were recruited as healthy controls. They gave their informed consent prior to participation. The experiments were performed with the approval of the Institutional Review Board of the Chang Gung Memorial Hospital, Taiwan, and National Hospital for Neurology and Neurosurgery in London, UK.

Blink reflex recovery cycle

Surface EMG recording Ag-AgCl electrodes at about 1-cm-diameter were placed bilaterally with the active electrode at the orbicularis oculi muscle

just below the lateral canthi and the reference electrode at the temporal region. Electric stimuli were given by a constant current generator (DS7A; Digitimer, Welwyn, UK) with electrodes attached over the right supraorbital nerve. Stimulation was given at an intensity of 2.5 times the sensory threshold, an intensity that was capable of producing a clear R1 and R2 component when a single stimulus was given. BR was tested on the right eye. Pairs of (conditioning followed by test) stimuli were given every 15 +/- 10% seconds at inter-stimulus intervals (ISIs) of 250ms, 500ms and 1000ms in a random order for 12 trials per condition.

Data Analysis

We measured the blink reflex recovery curve by calculating the R2 area ratio (the area of R2 evoked by test stimulation divided by the area of R2 evoked by conditioning stimulation) at each trial. The R2 area ratio was then averaged at each ISI. A two-way ANOVA was performed to compare the R2 area ratio at the three tested ISI (250, 500 and 100 ms) between all three subjects groups (MDYT1, NMDYT1 and control). The following two-way ANOVAs were done to compare each pair of the subject groups. SPSS 22.0 (SPSS for windows, IBM, USA) was used for statistical analysis. We

set statistical significant as P<0.05.

Result

A two-way ANOVA showed a significant difference between three groups (MDYT1, NMDYT1 and control) ($F(2,19)=7.53$, $p=0.004$) (Fig. 1). The further 2-way ANOVA analysis confirmed that was due to significant enhancement of the recovery of the R2 component of the blink reflex in MDYT1 and NMDYT1 as compared to controls ($F(1,15)=12.05$, $p=0.003$, $F(1,12)=6.998$, $p=0.021$, respectively). There was no difference between MDYT1 and NMDYT1 ($F(1,11)=1.663$, $p=0.224$), indicating that MDYT1 and NMDYT1 carriers have equivalent disinhibition in the blink reflex pathway in the brainstem.

Discussion

In our data, both MDYT1 and NMDY1 had abnormally enhanced blink reflex recovery curve as compared to healthy controls. Moreover, no statistical difference between manifesting and non-manifesting carriers suggests their brainstem circuits are equivalently affected by the DYT1 gene.

Abnormal blink reflex recovery curve suggests disinhibition the interneuronal pathway mediating the R2 component in blink reflex. Similar abnormality has been commonly reported in different forms of primary dystonia. [11] The central pathway of R2 response in the blink reflex is multisynaptic and involves several nuclei and tracts, including spinal trigeminal nucleus and laterobubal reticular formation, in the pons [21]. The current result suggests such R2 blink reflex pathway or the structures closely interact with it, e.g. pedunculopontine nucleus (PPN) [22], may be involved in the pathogenesis of dystonia in DYT1 carriers.

Previous studies have revealed that MDYT1 and NMDYT1 are both abnormal in the motor cortex. However, the abnormality pattern is different between manifesting and non-manifesting carriers. Although short interval intracortical inhibition (SICI) and cortical silent period were reduced in both MDYT1 and NMDYT1 as compared to healthy controls, SICI in MDYT1 was significantly less than that in NMDYT1 [8]. The two types of DYT1 carriers also responded differently to continuous theta burst stimulation and showed too much plasticity in MDYT1 and reduced plasticity in NMDYT1

[7]. Interestingly, at the spinal level, the 2nd & 3rd phases of reciprocal inhibition were reduced in manifesting carriers, while the reciprocal inhibition was normal in non-manifesting subjects [8]. Together with above results, the equal abnormality in the brainstem reflex in MDYT1 and NMDYT1 implies that the brainstem may therefore lie closer to the primary mechanism of DYT1 dystonia than the motor cortex.

Our finding is further support by a pathological study showing protein accumulation and inclusion bodies in cells located in the brainstem, but not in the cortex, cerebellum or basal ganglion or substantial nigra [20]. The perinuclear inclusion bodies mainly exist in the midbrain, periaqueductal gray (PAG), and pontine reticular formation (RF), and are also seen in the rostral pons like pedunculopontine nucleus (PPN), cuneiform nucleus (CN), and the griseum centrale mesencephali that are related with muscle tone control and mediate motor activities [20].

Functional neuroimaging studies indicated the ascending influence in the cerebellar-thalamo-cortical pathway in DYT1 gene carriers and mice model [4,5]. Some of the pathologically involved structure, e.g. PPN, received the

input information from cerebellum output flow and transport to basal ganglion via ascending pathway [24]. Furthermore, a study of eye blinking in dystonic patients with gene mutation in DYT1 discovered similar enhanced blinking reflex recovery but normal cerebellar function [12]. Therefore, it is reasonable to speculate that the brainstem dysfunction affects the ascending pathway to cause dystonia in DYT1 carriers. However, we cannot completely rule out the possibility that the brainstem disinhibition here was caused by the dysfunction of cerebellum.

Conclusion

In line with previous pathological findings, the present study revealed disinhibition in the brainstem of DYT1 carriers. Together with previous physiological and pathological results, the equal amount of dysfunction in clinically manifesting and non-manifesting carrier implies that the brainstem is likely at a level above the motor cortex and, probably, cerebellum and lies very close to the pathogenesis of dystonia in DYT1 gene carriers.

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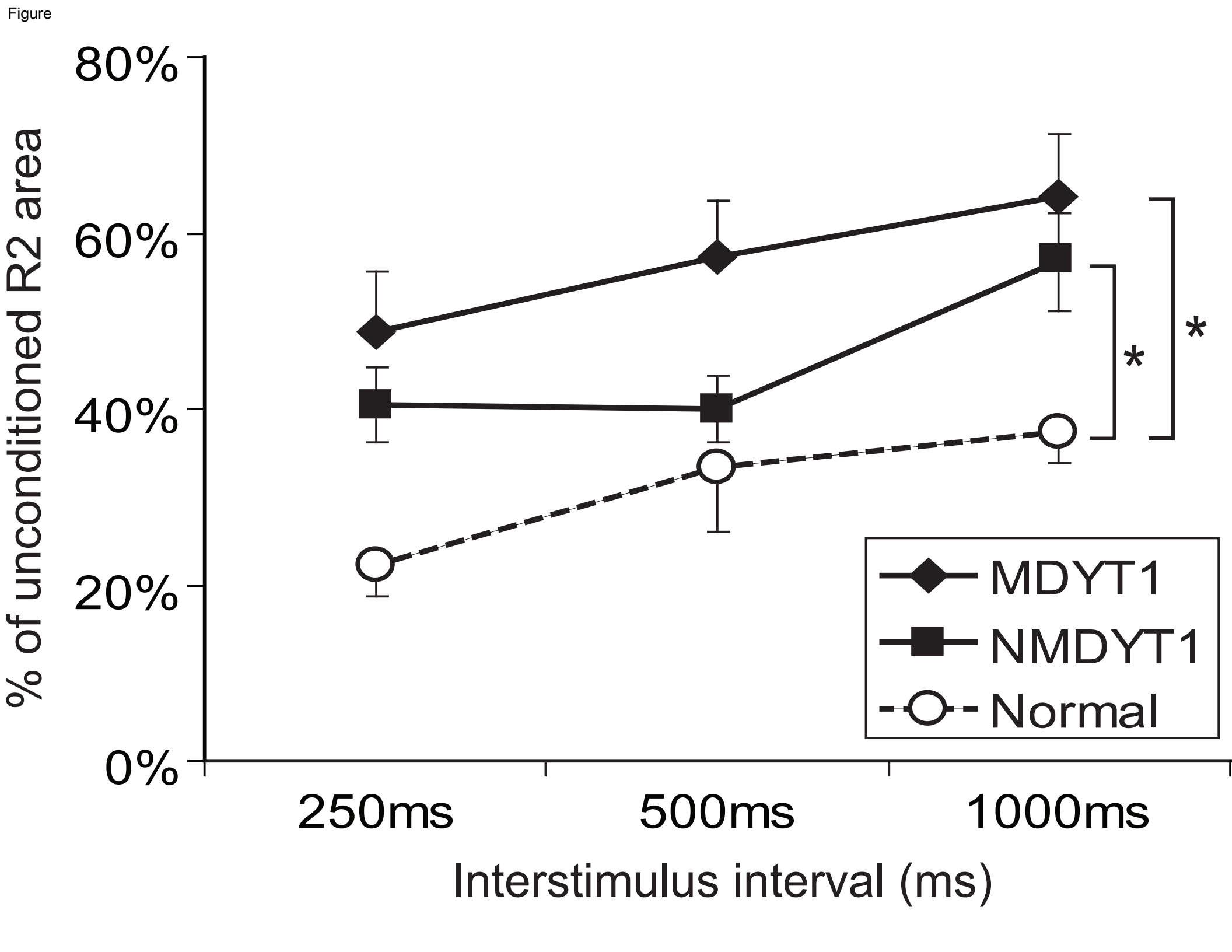
Figure Legend

Fig. 1. The blink reflex recovery curve in MDYT1, NMDYT1 and normal controls. Both MDYT1 and NMDYT1 groups had significant enhancement at the blink reflex recovery than the normal control group, while there was no difference between MDYT1 and NMDYT1 groups.

Figure


80%
60%
40%
20%
0%
% of unconditioned R2 area
250ms
500ms
1000ms
Interstimulus interval (ms)
MDYT1
NMDYT1
Normal
*
*