

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

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4 Po-Yu **Fong**^{a,b}, Mark J **Edwards**^c, Chin-Song **Lu**^{a,b,d}, Rou-Shayn **Chen**^{a,b,d},
5 John C **Rothwell**^e, Kailash P **Bhatia**^e, Ying-Zu **Huang**^{a,b,d,f}

6 a. Movement Disorder Division, Department of Neurology, Chang Gung
7 Memorial Hospital at Linkou, Taoyuan 333, Taiwan.

8 b. Neuroscience Research Center, Chang Gung Memorial Hospital at Linkou,
9 Taoyuan 333, Taiwan.

10 c. Department of Cell Sciences, St George's University of London, Cranmer
11 Terrace, London SW17 0RE, UK

12 d. School of Medicine, Chang Gung University , Taoyuan 333, Taiwan

13 e. Sobell Department of Motor Neuroscience and Movement Disorders, UCL
14 Institute of Neurology, London, WC1n 3BG, UK

15 f. Institute of Cognitive Neuroscience, National Central University, Taoyuan
16 320, Taiwan

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18
19 Corresponding Author:

20 Ying-Zu Huang, yzhuang@cgmh.org.tw

21 Chang Gung Memorial Hospital at Linkou

22 No.5, Fuxing St., Guishan Dist.

23 Taoyuan City 333

24 Taiwan

25 Phone: +886-3-3281200 EXT: 8414

26 FAX: +886-3-3285056

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29 Running head: Abnormal blink reflex recovery in DYT1

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38 **Abstract**

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

42

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

50

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

55

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

61

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

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70 **Key words:** DYT1, blink reflex, pathophysiology, dystonia

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74 **Introduction**

75 Dystonia is a kind of hyperkinetic movement disorder with clinical feature of
76 abnormal sustained limbs or trunk twisting posture. The neurophysiology
77 studies have revealed dysfunction in basal ganglion-sensorimotor network [1-
78 3], dysfunction in cerebellothalamocortical pathway [4,5], reduced cortical
79 inhibition with increased cortical plasticity [6-8], abnormal premotor-motor
80 connectivity [9,10] and decreased brain stem inhibition [1,3,11,12] and
81 reduced spinal cord reciprocal inhibition [13,14]. , Recent findings suggested
82 that dystonia could be a brain network disorder, and the basal ganglion may
83 not the primary source to develop the entire dysfunction network of dystonia
84 [15,16]. Hence, the exact pathogenesis of dystonia has been unclear so far.

85

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

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

105

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

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

113

114 **Method**

115 **Subjects**

116 We recruited eight DYT1 gene carriers (4 men and 4 women with average
117 age 46 ± 13.76) manifesting dystonia symptoms (MDYT1) and five carriers
118 (4 men and 1 woman with average 43.6 ± 15.43) without manifesting
119 dystonia symptoms (NMDYT1) from the movement disorder clinics at the
120 National Hospital for Neurology and Neurosurgery in London, UK and at
121 the Chang Gung Memorial Hospital at Linkou, Taiwan. Nine age-matched
122 healthy subjects (6 men, 3 women, average age 46 ± 7.05) were recruited
123 as healthy controls. They gave their informed consent prior to participation.

124 The experiments were performed with the approval of the Institutional
125 Review Board of the Chang Gung Memorial Hospital, Taiwan, and National
126 Hospital for Neurology and Neurosurgery in London, UK.

127

128 **Blink reflex recovery cycle**

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

131 just below the lateral canthi and the reference electrode at the temporal
132 region. Electric stimuli were given by a constant current generator (DS7A;
133 Digitimer, Welwyn, UK) with electrodes attached over the right supraorbital
134 nerve. Stimulation was given at an intensity of 2.5 times the sensory
135 threshold, an intensity that was capable of producing a clear R1 and R2
136 component when a single stimulus was given. BR was tested on the right
137 eye. Pairs of (conditioning followed by test) stimuli were given every 15 +/-
138 10% seconds at inter-stimulus intervals (ISIs) of 250ms, 500ms and
139 1000ms in a random order for 12 trials per condition.

140

141 **Data Analysis**

142 We measured the blink reflex recovery curve by calculating the R2 area
143 ratio (the area of R2 evoked by test stimulation divided by the area of R2
144 evoked by conditioning stimulation) at each trial. The R2 area ratio was
145 then averaged at each ISI. A two-way ANOVA was performed to compare
146 the R2 area ratio at the three tested ISI (250, 500 and 100 ms) between all
147 three subjects groups (MDYT1, NMDYT1 and control). The following two-
148 way ANOVAs were done to compare each pair of the subject groups. SPSS
149 22.0 (SPSS for windows, IBM, USA) was used for statistical analysis. We

150 set statistical significant as $P < 0.05$.

151

152 **Result**

153 A two-way ANOVA showed a significant difference between three groups

154 (MDYT1, NMDYT1 and control) ($F(2,19)=7.53$, $p=0.004$) (Fig. 1). The

155 further 2-way ANOVA analysis confirmed that was due to significant

156 enhancement of the recovery of the R2 component of the blink reflex in

157 MDYT1 and NMDYT1 as compared to controls ($F(1,15)=12.05$, $p=0.003$,

158 $F(1,12)=6.998$, $p=0.021$, respectively). There was no difference between

159 MDYT1 and NMDYT1 ($F(1,11)=1.663$, $p=0.224$), indicating that MDYT1

160 and NMDYT1 carriers have equivalent disinhibition in the blink reflex

161 pathway in the brainstem.

162

163 **Discussion**

164 In our data, both MDYT1 and NMDY1 had abnormally enhanced blink

165 reflex recovery curve as compared to healthy controls. Moreover, no

166 statistical difference between manifesting and non-manifesting carriers

167 suggests their brainstem circuits are equivalently affected by the DYT1

168 gene.

169

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

179

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

188 [7]. Interestingly, at the spinal level, the 2nd & 3rd phases of reciprocal
189 inhibition were reduced in manifesting carriers, while the reciprocal
190 inhibition was normal in non-manifesting subjects [8]. Together with above
191 results, the equal abnormality in the brainstem reflex in MDYT1 and
192 NMDYT1 implies that the brainstem may therefore lie closer to the primary
193 mechanism of DYT1 dystonia than the motor cortex.

194

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

203

204 Functional neuroimaging studies indicated the ascending influence in the
205 cerebellar-thalamo-cortical pathway in DYT1 gene carriers and mice model
206 [4,5]. Some of the pathologically involved structure, e.g. PPN, received the

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

215

216 **Conclusion**

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

223

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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

