ORIGINAL ARTICLE

All in the blink of an eye: new insight into cerebellar and brainstem function in DYT1 and DYT6 dystonia

A. Sadnicka, J. T. Teo, M. Kojovic, I. Pareés, T. A. Saifee, P. Kassavetis, P. Schwingenschuh, P. Katschnig-Winter, M. Stamelou, N. E. Mencacci, J. C. Rothwell, M. J. Edwards and K. P. Bhatia

Sobell Department of Motor Neuroscience and Movement Disorders, UCL Institute of Neurology, London, UK

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Background and purpose: Traditionally dystonia has been considered a disorder of basal ganglia dysfunction. However, recent research has advocated a more complex neuroanatomical network. In particular, there is increasing interest in the pathophysiological role of the cerebellum. Patients with cervical and focal hand dystonia have impaired cerebellar associative learning using the paradigm eyeblink conditioning. This is perhaps the most direct evidence to date that the cerebellum is implicated in patients.

Methods: Eleven patients with DYT1 dystonia and five patients with DYT6 dystonia were examined and rates of eyeblink conditioning were compared with age-matched controls. A marker of brainstem excitability, the blink reflex recovery, was also studied in the same groups.

Results: Patients with DYT1 and DYT6 dystonia have a normal ability to acquire conditioned responses. Blink reflex recovery was enhanced in DYT1 but this effect was not seen in DYT6.

Conclusions: If the cerebellum is an important driver in DYT1 and DYT6 dystonia our data suggest that there is specific cerebellar dysfunction such that the circuits essential for conditioning function normally. Our data are contrary to observations in focal dystonia and suggest that the cerebellum may have a distinct role in different subsets of dystonia. Evidence of enhanced blink reflex recovery in all patients with dystonia was not found and recent studies calling for the blink recovery reflex to be used as a diagnostic test for dystonic tremor may require further corroboration.

Introduction

Dystonia is a challenging group of syndromes to define pathophysiologically. Traditionally linked to dysfunction of the basal ganglia, more recent research defines dystonia as a network disorder within which the cerebellum may be an important node [1–4]. DYT1 and DYT6 are typically generalized dystonias with identified genes (TorsinA and THAP1). The case for cerebellar involvement is perhaps particularly strong in DYT1 dystonia due to the ability to investigate the disease using animal models which are increasingly refined in their ability to probe and implicate the cerebellum [5]. In humans, neuroimaging suggests that both DYT1 and DYT6 have reduced integrity of the cerebello-thalamo-cortical tract and metabolic cerebellar abnormalities have been identified in functional imaging studies [6]. Eyeblink conditioning (EBC) is a form of associative learning that has been shown to be critically dependent on the cerebellum in both animal [7] and human studies [8]. Patients with cervical and focal hand dystonia have lower rates of conditioning compared with controls [9], and this is perhaps the most direct evidence in humans that there is cerebellar dysfunction in focal dystonia.

In this study DYT1 and DYT6 dystonia were examined to determine if these patients also demonstrate impairments in EBC or changes in the blink

Correspondence: Dr M. Edwards, Box 146, National Hospital for Neurology and Neurosurgery, Queen Square, London WC1N 3BG, UK (tel.: +0044 203 4488749; fax: +0044 207 4191860; e-mail: m.j.edwards@ucl.ac.uk).

reflex recovery cycle (BRR) (a marker of brainstem excitability). These two paradigms provide a unique window into the function of the cerebellum and brainstem in these genetic dystonias.

Method

Eleven DYT1 and five DYT6 patients were recruited from the National Hospital for Neurology and Neurosurgery, London. Patients were individually age matched to controls as the ability to acquire EBC changes significantly with age [10]. All patients who received botulinum toxin injections were tested at least 3 months after their last treatment. Clinical details and medications are given in Table 1. The study was approved by the local ethics committee and written informed consent was obtained.

Electrical stimulation (square wave, 200 μs) of the supraorbital nerve was applied using chloride disc surface electrodes (cathode, right supraorbital foramen; anode, 2 cm above). Eyeblinks were captured by surface electromyography (EMG) electrodes over the right and left orbicularis oculi muscles and the signal was amplified (gain 2000), bandpass filtered (20–30 000 Hz), digitized (5 kHz) and stored for offline analysis using Cambridge Electronic Design (CED) 1401 hardware and Signal software (CED).

The EBC paradigm was identical to previous publications [9]. The conditioning stimulus (CS) was a loud (≈70 dB), 2000 Hz, 400 ms tone via binaural headphones. The unconditioned stimulus (US) was an electrical stimulus (200 μs, five times sensory threshold) to the supraorbital nerve at the termination of the CS which elicited a blink reflex (US). Repeated pairs of CS and US yielded conditioned blink responses (CRs) occurring before the US (Fig. 1a). EMG bursts were regarded as CRs if latency was >200 ms after onset of the CS but before the US. Six blocks of 11 trials (9 × CS-US, 1 × US and 1 × CS) were performed. US-only detects rates of spontaneous blinks and CS-only confirms that CRs are acquired independent of US. The seventh block measured extinction with 11 CS-only trials in which EMG bursts occurring 200–600 ms after the CS were considered CRs.

BRR was measured by applying pairs of electrical stimuli at five times sensory threshold to the supraorbital nerve at inter-stimulus intervals (ISIs) of 200, 300, 400 and 1000 ms [11] in a pseudo-randomized manner. The bilateral R2 component of the EMG response to the second stimulus is typically suppressed at ISIs of 200, 300 and 400 ms [11]. In subtypes of dystonia the lack of R2 suppression at these intervals is generally regarded as a marker of the increased brainstem excitability [12]. For each trial, EMG data from the non-stimulated left orbicularis oculi were rectified and the area ratio of the first and second R2 responses was calculated following subtraction of the mean pre-stimulus background activity (R2 duration × mean background activity). This was necessary to compensate for the higher levels of resting EMG in

Table 1 Clinical characteristics of patients

						Burk–Fahn–Marsden motor score									
		Age (years)	Dur (years)	Trem	Meds	Eyes 0–8	Mouth 0–8	S&S 0–16	Neck 0–8	RA 0–16	LA 0–16	RL 0–16	LL 0–16	Trunk 0–16	Total Max 120
DYT1	F	22	13	No	BTX	0	0	0	4	6	6	0	0	0	16
DYT1	F	30	19	No	Nil	0	0	0	2	6	4	4	0	0	16
DYT1	M	40	11	No	THP	0	0	0	8	0	0	0	0	4	12
DYT1	F	43	31	No	THP, CLZ	0	4	8	6	2	4	6	6	6	42
DYT1	M	44	7	No	Nil	0	0	1	6	0	0	4	1	9	21
DYT1	F	47	39	Yes	THP, CLZ	0	0	0	4	9	4	4	0	1	22
DYT1	M	49	35	Yes	Nil	0	0	0	4	12	12	0	0	6	34
DYT1	M	50	30	No	BTX, THP	0	6	1	8	12	12	4	4	8	55
DYT1	F	67	56	Yes	THP, CLZ	0	0	0	6	12	12	9	9	9	57
DYT1	F	72	70	Yes	BTX	0	0	2	0	0	12	4	12	0	30
DYT1	M	81	46	Yes	Nil	0	6	0	6	12	8	6	2	4	44
DYT6	F	23	14	Yes	THP	0	8	8	6	2	4	6	6	6	46
DYT6	F	26	19	No	BTX, THP	0	0	1	6	0	0	4	1	9	21
DTY6	F	34	24	No	BTX	0	0	0	4	6	6	0	0	0	16
DTY6	F	36	33	No	Nil	0	0	0	8	0	0	0	0	4	12
DTY6	M	66	49	No	BTX, THP	0	6	0	6	12	8	6	2	4	44

Dur, duration of disease at time of testing; Trem, tremor; Meds, medications at time of study; BTX, botulinum toxin injections; THP, trihexyphenidyl; CLZ, clonazepam; S&S, speech and swallowing; RA, right arm; LA, left arm; RL, right leg; LL, left leg; Max, maximum possible total score of Burk–Fahn–Marsden motor score is 120. Topography represented with 'hot' shading (red indicating severely affected). None of the patients had blepharospasm.

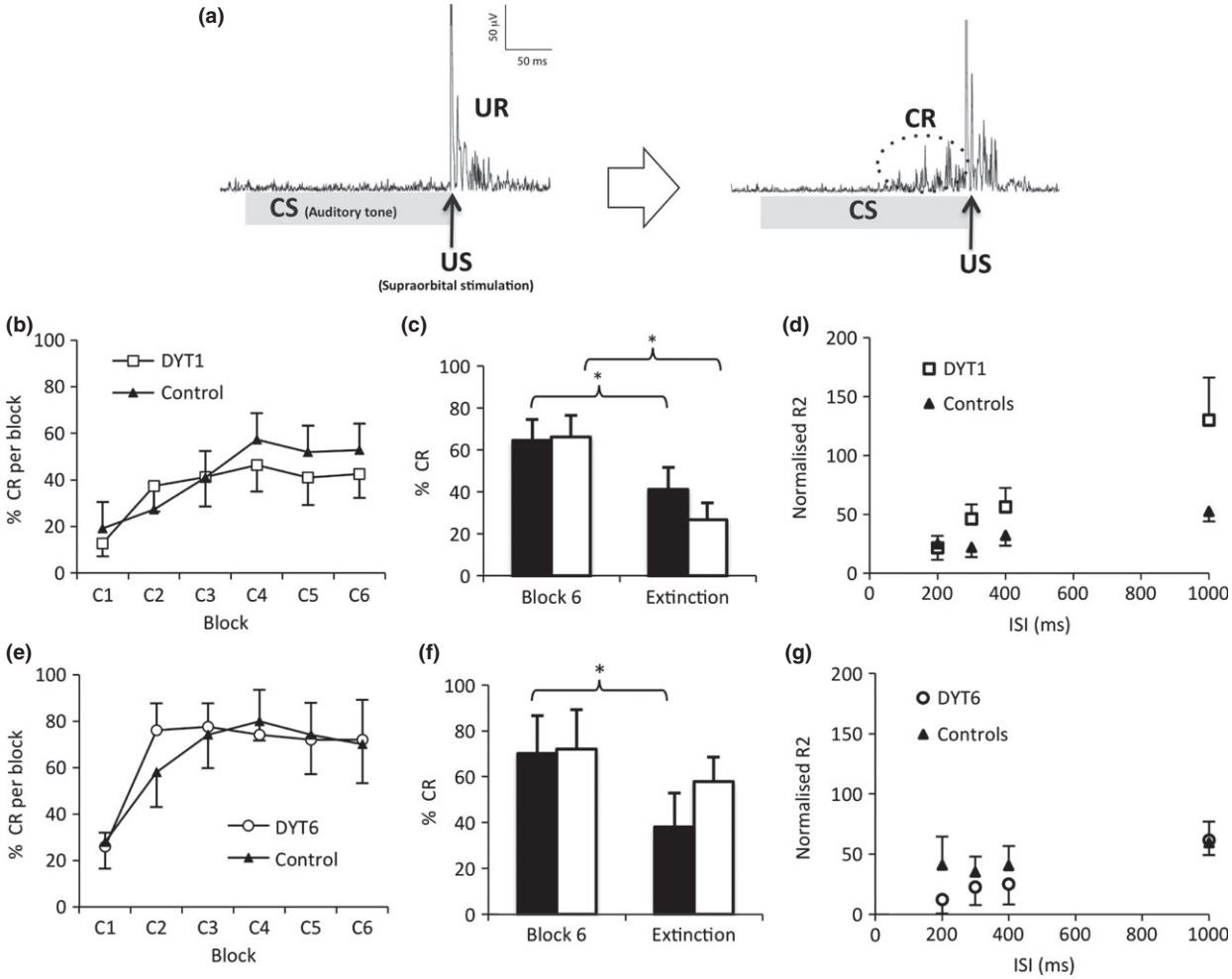

Figure 1 (a) Rectified EMG traces from orbicularis oculi demonstrating the EBC paradigm and responses before and after conditioning has developed. See methods section for explanation of abbreviations. (b) EBC over six conditioning blocks comparing DYT1 to controls. (c) evidence of extinction in controls (black bars) and DYT1 (clear bars), (d) BRR at different ISIs demonstrated a different time profile of inhibition in DYT1 compared to controls with greater recovery of R2 at later ISIs. (e) EBC over six conditioning blocks in DYT6 and controls. (f) evidence of extinction in controls (black bars) but not DYT6 (clear bars). (g) No difference in BRR between DYT6 and controls.

patients with dystonia of the face. Mean values were calculated from the eight trials for each ISI.

Rates of CRs during EBC were assessed using repeated measures ANOVA (SPSS for Windows, version 21; IBM, New York, NY, USA) with block as the within-subject factor (block 1–6) and group as the between-subjects factor (dystonia, normal). Extinction rates in subjects who successfully conditioned (defined as >40% CRs in any block) were examined using paired Student t tests comparing the percentage of CRs in block 6 to the percentage of CRs in the extinction block. BRR was assessed using repeated measures ANOVA with ISI as the within-subject factor (200, 300, 400, 1000 ms) and group (dystonia, normal) as the between-subjects factor. The Greenhouse–Geisser method was used to correct for non-sphericity and Bonferroni correction was used with multiple comparisons. Otherwise statistical significance was $P < 0.05$.

Results

Patients with DYT1 dystonia (Fig. 1b–d) had comparable rates of conditioning to controls: effect of block $F(3.16, 63.3) = 11.62$, $P < 0.001$; but no block × group interaction $F(3.16, 63.3) = 1.17$, $P = 0.33$, or effect of group $F(1, 20) = 0.128$, $P = 0.725$. Seven DYT1 patients and nine controls acquired the CR to a level of 40%. Both patients ($P = 0.0068$) and controls ($P = 0.0094$) exhibited extinction.

Eyeblink conditioning was also comparable in patients with DYT6 dystonia (Fig. 1e–g) and controls: effect of block $F(5, 40) = 15.4$, $P < 0.001$; but no effect of block × group $F(5, 40) = 0.752$, $P = 0.60$, or group $F(1, 8) = 0.16$, $P = 0.903$ (Fig. 1e). The fact that all patients with DYT6 conditioned to high levels, despite the small group numbers, suggests that there is no impairment in acquisition of EBC in this genetic group. All DYT6 patients and controls acquired CR to a level of 40%. Unlike controls ($P = 0.030$), DYT6 patients failed to show significant extinction ($P = 0.525$).

Although EBC appears to differ between DYT1 and DYT6, this is likely to be due to a greater proportion of younger people in the DYT6 group (80% of DYT6 patients <40 years old, 18% of DYT1 patients). The ability to acquire EBC is critically dependent on age [10] and therefore a comparison between the dystonia groups was not performed. A majority of patients in both groups were receiving treatment for dystonia when the study was performed (oral medications or botulinum toxin injections). It is proposed that EBC is within normal limits in both genetic groups. As none of these treatments should enhance the ability to acquire EBC [13,14], the fact that patients were receiving treatment is not thought to be obscuring a deficit in EBC.

One DYT1 patient, one DYT6 patient and two controls did not complete BRR as they found the paradigm uncomfortable. BRR differed between DYT1 patients and controls: effect of ISI $F(1.56, 25.0) = 12.0$, $P = 0.001$, and ISI × group $F(1.56, 25.0) = 3.83$, $P = 0.045$, but no effect of group $F(1, 16) = 2.11$, $P = 0.166$. *Post hoc* analysis did not show significant differences at an individual ISI. No difference in BRR was seen between DYT6 patients and controls: effect of ISI $F(1.41, 9.86) = 5.15$, $P = 0.038$, but not ISI × group $F(1.41, 9.85) = 0.828$, $P = 0.425$, or group $F(1, 7) = 0.432$, $P = 0.532$.

Discussion

In this study it is shown that patients with DYT1 and DYT6 have EBC rates comparable with controls. In addition, patients with DYT1 have differences in their BRR to controls, which suggests reduced inhibition within brainstem circuits, and this effect was not observed in DYT6.

Eyeblink conditioning is critically dependent on intact olivo-cerebellar function, and is abnormal in patients with focal hand and cervical dystonia [9,15]. The normal EBC seen in DYT1 and DYT6 is thus in contrast to these focal dystonias and suggests that the different forms of primary dystonia may have different neuroanatomical correlates. It is intriguing to hypothesize what this signifies. Most obviously our data may have their origins in the phenotypical differences associated with subtypes of dystonia. The genetic background of most focal dystonias is still unknown, the disease occurs later in life and there is a greater influence of environment factors. Perhaps the cerebellum takes a greater compensatory role in focal dystonia to counteract the dystonic motor activity and due to competing demands on its net function this impairs the ability of the cerebellar networks to acquire CRs. However, this is unlikely to be the whole story. EBC is normal in patients with secondary dystonia [16] caused by basal ganglia lesions, arguing against a straightforward compensatory role of the cerebellum in alleviating symptoms of dystonia.

Our results are surprising as evidence in support of cerebellar deficits in DYT1 dystonia, in particular, is perhaps stronger than for focal dystonia. Whilst histological studies do not demonstrate clear structural abnormalities of the cerebellum in humans with DYT1 dystonia [17], or in rodent models [5], subtle microstructural defects (such as thinner dendrites and fewer dendritic spines) are observed in the Purkinje cells of DYT1 mouse models [18]. Furthermore, by reducing cerebellar output by knocking out Purkinje cells, motor symptoms improve in a DYT1 knock-in animal model [19]. As yet there are no animal models of DYT6. However, the gene is widely expressed in the central nervous system including cerebellar neurons [6] and neuropathological changes such as reduced Purkinje cell density in post-mortem studies of humans with cervical dystonia have been linked to THAP1 sequence variations [20]. Human imaging studies using fractional anisotropy demonstrate reduced integrity of the cerebello-thalamo-cortical pathway in patients with DYT1 and DYT6 dystonia. Other studies have examined cerebellar functional activity during the motor task of sequence learning. Interestingly a genotypic effect was found, such that carriers of the DYT1 mutation (both manifesting and non-manifesting) had impaired sequence learning and an associated excessive activation of the left lateral cerebellar cortex whereas DYT6 carriers (irrespective of clinical penetrance) did not demonstrate the impairment in sequence learning or the abnormal cerebellar activation [21].

The absence of clinical signs of cerebellar dysfunction in patients with primary dystonia highlights that if the cerebellum is implicated in the pathophysiology it is likely to be a selective impairment of a pertinent feature of motor control. Our patients with genetic dystonia, at least in the circuits essential to

EBC, seem to have normal cerebellar function. Furthermore this type of associative learning with its clear dependence on recognizing salient sensory inputs within millisecond timing intervals is not impaired.

The BRR in DYT1 patients showed hyperexcitability (Fig. 1d) in line with previous experimental results that have tested the BRR in generalized dystonia, segmental dystonia, focal cervical dystonia and dystonic hand tremor [9,12,22]. In contrast DYT6 patients did not differ from controls (Fig. 1g), a finding which has previously been observed in focal arm and hand dystonias [9,12]. This was surprising as the DYT6 patients had clinical involvement of cranio-cervical muscles (although none had blepharospasm) and this has previously been thought to be a factor in determining the extent of abnormal brainstem interneuron function [12]. Our findings add further complexity to the need to define a specific electrophysiological abnormality of the BRR and its functional significance in dystonia pathophysiology. The BRR is also disinhibited during voluntary eye musculature contraction in healthy controls, peripheral disorders that evoke facial muscle contractions and other movement disorders [23–25]. It is currently unclear what the differences in BRR profiles across the subtypes of dystonia signify. Recently the BRR has shown surprising ability (100% sensitivity and specificity) to dissociate between tremor subtypes that have been first classified clinically as dystonic tremor or essential tremor [22] and has been proposed as a potential test for dystonic tremor. Our results and those of others suggest that caution should be taken when proposing a single electrophysiological abnormality as diagnostic of dystonia.

The main limitation of our study is the small number of available subjects with DYT6 dystonia which reflects the lower prevalence of this genetic dystonia and further multicentre studies are encouraged.

Conclusions

The cerebellum has received increasing attention as an important neuroanatomical structure involved in the pathophysiology of dystonia. However, this research is still at an early stage and it remains difficult to obtain direct evidence in humans to specifically implicate the cerebellum in dystonia. Our data suggest that the circuits involved with EBC within the cerebellum maintain normal function in DYT1 and DYT6 dystonia. Thus, current disease models discussing dystonia from a neuroanatomical perspective need to incorporate emerging differences between subtypes of primary dystonia and be able to explain any results that currently appear at odds across animal and human research modalities.

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References

1. Neychev VK, Gross RE, Lehericy S, Hess EJ, Jinnah HA. The functional neuroanatomy of dystonia. *Neurobiol Dis* 2011; **42:** 185–201.
2. Prudente CN, Hess EJ, Jinnah HA. Dystonia as a network disorder: what is the role of the cerebellum? *Neuroscience* 2013; **260C:** 23–35.
3. Sadnicka A, Hoffland BS, Bhatia KP, van de Warrenburg BP, Edwards MJ. The cerebellum in dystonia – help or hindrance? *Clin Neurophysiol* 2012; **123:** 65–70.
4. Filip P, Lungu OV, Bares M. Dystonia and the cerebellum: a new field of interest in movement disorders? *Clin Neurophysiol* 2013; **124:** 1269–1276.
5. Oleas J, Yokoi F, Deandrade MP, Pisani A, Li Y. Engineering animal models of dystonia. *Mov Disord* 2013; **28:** 990–1000.
6. Argyelan M, Carbon M, Niethammer M, *et al*. Cerebellothalamocortical connectivity regulates penetrance in dystonia. *J Neurosci* 2009; **29:** 9740–9747.
7. Thompson RF. The neurobiology of learning and memory. *Science* 1986; **233:** 941–947.
8. Gerwig M, Kolb FP, Timmann D. The involvement of the human cerebellum in eyeblink conditioning. *Cerebellum* 2007; **6:** 38–57.
9. Teo JT, van de Warrenburg BP, Schneider SA, Rothwell JC, Bhatia KP. Neurophysiological evidence for cerebellar dysfunction in primary focal dystonia. *J Neurol Neurosurg Psychiatry* 2009; **80:** 80–83.
10. Solomon PR, Pomerleau D, Bennett L, James J, Morse DL. Acquisition of the classically conditioned eyeblink response in humans over the life span. *Psychol Aging* 1989; **4:** 34–41.
11. Kimura J, Harada O. Recovery curves of the blink reflex during wakefulness and sleep. *J Neurol* 1976; **213:** 189–198.
12. Nakashima K, Rothwell JC, Thompson PD, *et al*. The blink reflex in patients with idiopathic torsion dystonia. *Arch Neurol* 1990; **47:** 413–416.
13. Robinson L, Platt B, Riedel G. Involvement of the cholinergic system in conditioning and perceptual memory. *Behav Brain Res* 2011; **221:** 443–465.
14. Garcia KS, Mauk MD. Pharmacological analysis of cerebellar contributions to the timing and expression of conditioned eyelid responses. *Neuropharmacology* 1998; **37:** 471–480.
15. Hoffland BS, Kassavetis P, Bologna M, *et al*. Cerebellum-dependent associative learning deficits in primary dystonia are normalized by rTMS and practice. *Eur J Neurosci* 2013; **38:** 2166–2171.
16. Kojovic M, Parees I, Kassavetis P, *et al*. Secondary and primary dystonia: pathophysiological differences. *Brain* 2013; **136:** 2038–2049.
17. Standaert DG. Update on the pathology of dystonia. *Neurobiol Dis* 2011; **42:** 148–151.
18. Song CH, Bernhard D, Hess EJ, Jinnah HA. Subtle microstructural defects of the cerebellum in a knock-in mouse model of DYT1 dystonia. *Neurobiol Dis* 2013; **62:** 372–380.
19. Yokoi F, Dang MT, Li Y. Improved motor performance in Dyt1 DeltaGAG heterozygous knock-in mice by cerebellar Purkinje-cell specific Dyt1 conditional knocking-out. *Behav Brain Res* 2012; **230:** 389–398.
20. Prudente CN, Pardo CA, Xiao J, *et al*. Neuropathology of cervical dystonia. *Exp Neurol* 2013; **241:** 95–104.
21. Carbon M, Argyelan M, Ghilardi MF, *et al*. Impaired sequence learning in dystonia mutation carriers: a genotypic effect. *Brain* 2011; **134**(Pt 5)**:** 1416–1427.
22. Nistico R, Pirritano D, Salsone M, *et al*. Blink reflex recovery cycle in patients with dystonic tremor: a cross-sectional study. *Neurology* 2012; **78:** 1363–1365.
23. Yaman M, Sahin S, Kiziltan ME. Blink reflex recovery in central and peripherally originated movement disorders of the cranio-cervical area: a comparative study. *Electromyogr Clin Neurophysiol* 2009; **49:** 19–25.
24. Sommer M, Wobker G, Ferbert A. Voluntary eyelid contraction modifies the blink reflex recovery cycle. *Acta Neurol Scand* 1998; **98:** 29–35.
25. Kimura J. Disorder of interneurons in Parkinsonism. The orbicularis oculi reflex to paired stimuli. *Brain* 1973; **96:** 87–96.