Impaired Eye Blink Classical conditioning distinguishes dystonic patients with and without tremor.

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Conflict of interest

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Abstract

Background and objectives: Tremor is frequently associated with dystonia, but its pathophysiology is still unclear. Dysfunctions of circuits involving the cerebellum are known to play a role in the pathophysiology of action-induced tremors, and therefore here we tested if a cerebellar dysfunction might explain the pathophysiology of tremor even in dystonia.

Methods: We studied the brainstem function by measuring the R2 blink reflex recovery cycle (BRR) and and the cerebellar function by applying the paradigm of eye blink classical conditioning (EBCC) in 25 patients with primary (isolated) dystonia, with and without tremor. We compared the results with those obtained in a group of age-matched healthy controls.

Results: Statistical analysis did not disclose any significant clinical difference among dystonic patients with and without tremor. Patients with dystonia (regardless the presence of tremor) had a significantly increased brainstem excitability when compared with healthy controls. Finally, when analysing the number of conditioned responses (CRs) we found that total number of CRs was significantly decreased in dystonic patients with tremor when compared either with dystonic patients without tremor either with healthy controls. Dystonic patients without tremor on the contrary did not differ from healthy controls in number of CRs.

Conclusions: Functional cerebellar impairment segregates with the presence of tremor in patients with dystonia, implying that cerebellum may have a role in the occurrence of tremor in dystonia.

Introduction

Dystonia is the result of network impairment,[1] within which the cerebellum has been recently suggested to represent a crucial node.[2] Eye blink classical conditioning (EBCC) is a form of predictive learning, related to the integrity of the olivo-cerebellar circuit.[3]

While EBCC has been reported to be normal in patients with generalized dystonia due to *TOR1A* (DYT1) and *THAP1* (DYT6) mutations,[4] as well as in patients with secondary dystonia,[5] patients with different types of isolated focal dystonia have been found to have lower rates of classical conditioning when compared with healthy controls.[5-7] However, in these studies the presence or absence of associated tremor has been overlooked. Tremor is in fact a common feature of dystonia,[8,9] its prevalence ranging from 11% to 87%.[9] The pathophysiology of tremor in dystonia is still largely elusive[9] and it is possible that a cerebellar dysfunction contributes to its development. Dysfunctions of circuits involving the cerebellum and inferior olives have been in fact reported to play a critical role in the pathophysiology of action-induced tremors,[10] and EBCC has been also reported to be impaired in patients with essential tremor(ET)[11] and neuropathic tremor.[12]

Therefore, our aim was to test whether a cerebellar dysfunction segregates with the presence of tremor in patients with dystonia. In order to address this issue we have here tested EBCC in patients with isolated focal dystonia, with and without associated tremor.

Methods

Subjects and clinical evaluation

We prospectively recruited 25patients with primary (isolated) cervical dystonia (# 13 with tremor and # 12 without tremor) among those attending the Movement Disorders outpatients' clinic at the National Hospital for Neurology and Neurosurgery, London. Clinical features were evaluated by means of neurological examination and review of clinical notes in order to exclude patients

with known genetic mutations or acquired dystonia. The local ethics committee approved this study. All participants gave informed written consent prior to the study.

Clinical assessment included: the scale for the assessment and rating of ataxia (SARA scale),[13] (the Fahn-Tolosa-Marin Tremor Rating Scale (TRS)[14]for the rating of tremor and the Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS)[15]for assessing the severity of cervical dystonia.

Neurophysiological study

Neurophysiological investigations were conducted by a neurophysiologist blinded to the clinical diagnosis and included the R2 blink reflex recovery cycle (BRR) and the EBCC.

The BRC and the EBCC were assessed in all subjects according to a previously described protocol.[6] Briefly, to record the BRC, the right supraorbital nerve was stimulated with square wave pulses of 200μs width. Single or double pulses were given randomly at interstimulus intervals (ISI) of 200, 300, 400 and 1000 ms. Six trials for each ISI were collected. Surface electromyographic (EMG) activity was recorded from the right and left orbicularis oculi muscles. Recorded activity was DC-corrected, rectified, and averaged; finally, the ratio between unconditioned and conditioned R2 area was calculated.

For the EBCC, the conditioning stimulus (CS) was a loud (70-80 dB; 2000 Hz) tone lasting 400 ms, delivered via binaural headphones. The CS inconsistently produced an acoustic startle response ("alpha blink") occurring within 200 ms after the CS. The unconditionted stimulus (US) was a square electrical pulse of 200 μs length and of intensity equal to five times the sensory threshold, delivered 400 ms after the CS over the right supraorbital nerve. Surface EMG was recorded bilaterally from the right orbicularis oculi. Pairs of CS and US at 400 ms intervals were delivered in 6 acquisition blocks (each consisting of 9 CS-US pairs, 1 US only, 1 CS only trial). A seventh block consisted of 11 CS-only trials to measure extinction.

EMG bursts were regarded as “alpha blinks” if their amplitude exceeded 50 μV and if their latency was less than 200 ms after the CS. In CS-US pairs, EMG bursts were regarded as conditioned responses (CRs) if latency was at least 200 ms after the CS but before the US. For the trial including only the CS, EMG bursts occurring 200–600 ms after the CS were considered CRs.

Statistical analysis

To assess R2 recovery, we used a mixed ANOVA with “group” (dystonia without tremor, dystonia with tremor, healthy subjects) and“ISI” (200, 300, 400 and 1000 ms) as factor of analysis.

To compare the number of CRs summed over all blocks and in each block (from 1 to 7) in the three groups we used several Kruskal-Wallis tests. Mann-Whitney tests were used to assess differences among groups (dystonia without tremor, dystonia with tremor and healthy subjects) in significant blocks.

Clinical differences between patients with and without dystonia were assessed with several Mann-Whitney U tests, while two one-way ANOVAs were used to compare sensory threshold and intensity for evoking blink reflex in all groups. Possible correlations among demographic data, clinical features (disease duration, TRS and TWSTRS) and neurophysiological results in the two groups of patients were evaluated with the Spearman’s rank correlation coefficient. Normal distribution of data was assessed by means of Shapiro-Wilks’ test. Greenhouse Geisser correction was used when necessary to correct for non sphericity (i.e Mauchly's test < 0.05). All p values < 0.05 were considered significant. Levene’s test was used to assess homogeneity of variance across groups. Bonferroni post-hoc test was used for post-hoc analyses following the ANOVA. Bonferroni correction was used to correct for multiple comparisons.

Results

Based on clinical examination and video-reviewing, dystonic patients were categorized as with tremor (n=13) and without tremor (n=12). Among patients with tremor, 9 had dystonic tremor (DT) and 4 had both dystonic tremor and tremor associated with dystonia (TAWD), as per consensus classification.[16] Statistical analysis did not disclose significant clinical difference among patients with and without tremor as to age, disease duration and severity of dystonia (Table).

Blink reflex excitability (Figure 1)

Unpaired t-tests showed comparable sensory thresholds and intensities for eliciting blink reflex in all groups (P>0.05 for all comparisons).The mixed ANOVA showed a significant effect of "group" ($F_{2,34}$= 12.35; P<0.001),"ISI"($F_{1.5,52.4}$=51.54; P<0.001) and a significant interaction of "group x ISI" ($F_{3.1,52.37}$=2.53; P=0.025). Post hoc analyses showed that patients with and without tremor did not differ in any of the ISI considered (P>0.005 in all comparisons), while there was a significant effect at all ISI when comparing both groups of patients with healthy subjects (P<0.05 in all comparisons).

Eyeblink classical conditioning (Figure 2)

Unpaired t-tests showed comparable sensory threshold and intensity for eliciting blink reflex in all groups (P>0.05 for all comparisons).

Kruskal-Wallis tests showed a significant difference in CRs summed over all blocks among the three groups (H2=8,49, P=0,014). Particularly Mann-Whitney tests showed that healthy subjects and patients with dystonia had the same total number of CRs (P>0.05), while patients with dystonia and tremor differed in total CRs if compared both with healthy subjects (Z=-2.96, P<0.05) and with patients with dystonia without tremor (Z=1.97, P<0.05).

Considering the rate of CRs over different blocks, Kruskal-Wallis tests showed a significant difference among the three groups in Block 3 (H_2=6.92, P=0.028), Block 4 (H_2=9.19, P=0.007), Block 5 (H_2=11.35, P=0.002) and Block 6 (H_2=8.42, P=0.012). Mann-Whitney tests showed that patients without tremor and healthy subjects did not differ regarding CRs in all blocks considered (P>0.05 in all comparisons) while patients with tremor and healthy subjects differed in Block 3 (Z=-2.87, P<0.05), Block 4 (Z=-3.27, P<0.05), Block 5(Z=-3.32, P<0.01) and Block 6 (Z=-2.92, P<0.05). Finally, patients without tremor and patients with tremor differed in conditioning in Block 5 (Z=-2.36, P<0.05) and Block 6 (Z=-2.01, P<0.05).

Correlation of BRR and EBCC with clinical features

Spearman's rank correlation coefficient did not disclose any correlation between neurophysiological results and clinical data (disease duration, TRS and TWSTRS) (all P> 0.05).

Discussion

Our results show that ~~patients with dystonia and tremor differ from patients without tremor with regards to the cerebellar function. In fact,~~ in the EBBC paradigm, patients with tremor had lower rates of CRs ~~when~~ compared ~~to~~ with both healthy controls and non-tremulous patients. Given that ~~The~~ EBCC ~~is a form of simple and well-known paradigm of associative learning that~~ is strongly ~~cerebellar~~ dependent on the cerebellum~~.~~[3] the data is consistent with the notion that tremor in dystonia may be linked to abnormalities in cerebellar circuits. We note however, that ~~There are some suggestions that~~ this type of associative learning may be also influenced by the brainstem eyeblink reflex centres ~~of the eyeblink reflex~~ via the red nucleus,[17] along with the pontine nuclei and the inferior olive. However, we think it is unlikely that such abnormalities are present in any significant degree in our~~in our~~ population ~~it is unlikely that abnormal brainstem inhibition has~~

~~determined decreased conditioned responses in the tremulous group given that~~ since all~~both~~ groups showed a similar degree of brainstem hyperexcitability, as measured by means of the BRC. ~~On this basis, we would support a specific role of the cerebellum in the development of tremor in patients with dystonia.~~

The hypothesis of cerebellar involvement ~~hypothesis~~ in dystonia has increasingly gained momentum based on a body of clinical, neurophysiological and imaging evidence. However, contrasting results have been obtained with regards to the EBCC. ~~In fact, the~~ EBCC has been reported to be impaired in different types of primary focal dystonia,[6] but not in patients with hereditary dystonia due to *TOR1A* or *THAP1* mutations[4] or in secondary dystonia.[5] Such a discrepancy might be ~~owing~~ due to the fact that different pathophysiological mechanisms ~~might be~~ are responsible for different types of dystonic syndromes. ~~On~~ In this regard, there are no studies specifically addressing the prevalence of tremor in hereditary or acquired dystonia, however the occurrence of tremor in early onset dystonia seems to be lower, while tremor is more frequently reported in late onset idiopathic dystonia, ~~and~~ particularly in segmental (craniocervical) and cervical dystonia.[9] Moreover, the presence of tremor has been overlooked in previous ~~researches~~ studies and this might, on its own, explain the~~se~~ contradictory results. ~~In fact,~~ ~~i~~Impaired EBCC has been reported in other types of tremor, including ET[11] and neuropathic tremor from acquired disease,[12] and the cerebello-thalamo-cortical pathway has been generally deemed to be implicated in the pathophysiology of tremor.

The pathophysiological mechanisms of dystonic tremor have not yet been clarified. Some authors have suggested that it might be linked to abnormal oscillatory activity in the internal globus pallidus (GPi).[18] However, ~~despite~~ although deep brain stimulation (DBS) of the GPi ameliorates dystonic symptoms, it does not always improve tremor, suggesting that ~~other~~ structures beyond the GPi ~~may account for the occurrence of~~ are involved in producing tremor in dystonia.

Interestingly, thalamic DBS, particularly of the ventral intermediate nucleus (e.g., the main target of cerebellar projections to the thalamus) improves tremor in dystonic patients.[19,20] Moreover, anatomopathological abnormalities involving the cerebellum have been reported in dystonic patients with additional tremor.[21] Hence, our results ~~together with the above body of~~ add to this evidence ~~would~~ and suggest that the development of tremor in patients with dystonic syndromes involves the cerebellum. The absence of clinical cerebellar signs in our population confirms previous findings[2] and points toward a selective impairment of a discrete feature of motor control of the cerebellum. Interestingly, conditioned responses have been shown to increase after non-invasive cerebellar stimulation in patients with dystonia,[7] suggesting that cerebellar abnormalities are dynamic and ~~likely occurring~~may occur at a functional level.

~~In keeping with the model suggesting dystonia as a network disorder, our study points towards a functional cerebellar impairment as major determinant for the occurrence of tremor in patients with dystonia.~~

These results ~~would be important~~are of interest not only ~~for~~ from a pathophysiological ~~inferences~~viewpoint, but also for their potential treatment implications. In fact, it might be possible that patients with dystonia and tremor would benefit from chemical, functional or surgical approaches targeting the cerebellum along with the basal ganglia.

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Table. Clinical features of dystonic patients.

Legend: Av: average; Sd: standard deviation; F: female; M: male; TWSTRS: Toronto Western Spasmodic Torticollis Rating Scale; SARA: scale for the assessment and rating of ataxia

Figure 1

Blink recovery cycle in healthy subjects and patients with dystonia (with and without tremor).

Vertical bars indicate standard error.

Figure 2

Eyeblink classical conditioning in healthy subjects and patients with dystonia (with and without tremor). Mean of conditioned responses (CRs) over the six acquisition blocks is shown on the right side of the Figure. Error bars represent standard error.