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Neurophysiological evidence for cerebellar dysfunction in primary focal dystonia

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ABSTRACT

Recent studies have suggested that there may be functional and structural changes in the cerebellum of patients with adult onset primary focal dystonia. The aim of this study was to establish whether there is any neurophysiological indicator of abnormal cerebellar function, using the classic eyeblink conditioning paradigm. This paradigm at short intervals is dependent on the olivo-cerebellar circuit and does not require cerebral and basal ganglia structures. Eyeblink conditioning was performed by pairing an auditory tone with a supraorbital nerve stimulus with a delay interval of 400 ms in 12 patients with primary focal dystonia (seven cervical dystonias, five focal hand dystonias) and eight healthy controls. Healthy controls produced more conditioned eyeblink responses than patients with focal dystonia, indicating an abnormality of associative learning in this patient population. This study provides neurophysiological evidence for functional changes in the olivo-cerebellar pathway of patients with primary focal dystonia. Further work needs to be done to determine if these changes are primary, secondary or epiphenomenal to the disease.

Recent voxel based morphometry studies have shown structural grey matter abnormalities in the cerebellum in patients with upper limb dystonia,[1] cervical dystonia[2 3] and blepharospasm.[2] Furthermore, there are reports of cerebellar lesions producing dystonia in humans,[4] and dystonia is associated with cerebellar atrophy.[5 6] Functional imaging studies have shown increased activation in the cerebellum in writer's dystonia,[7] blepharospasm[8] and DYT1 dystonia,[9] all this providing evidence for the cerebellum playing a role in a condition traditionally thought to be related to the basal ganglia.

Eyeblink classical conditioning is a well characterised experimental paradigm that is highly conserved across species and is dependent on the cerebellum.[10] It has been demonstrated to occur in decerebrate animals, indicating that only brainstem (inferior olive) and cerebellar structures are needed for this paradigm.[11] The paradigm consists of pairing a weak conditioning stimulus (CS) with a strong unconditioned stimulus (US) repeatedly. After repeated pairings, healthy humans produce conditioned responses (CRs) consisting of an eyeblink starting before the US. Patients with cerebellar atrophy are less able to produce CRs[12] as are patients with superior cerebellar infarction[13] and patients with essential tremor.[14] Significantly, patients with Parkinson's disease perform as well as healthy controls,[15] indicating that nigrostriatal degeneration and basal ganglia dysfunction do not have a significant impact on this learning paradigm.

Thus we sought to establish if eyeblink classical conditioning was abnormal in patients with adult onset focal dystonia.

METHODS

Subjects

Twelve patients with adult onset focal dystonia were recruited. Seven patients had cervical dystonia (spasmodic torticollosis) and five patients had focal, task specific hand dystonia (three writer's dystonia, two musician's dystonia). Patient details are summarised in table 1. Eight healthy age matched controls were also recruited (mean age 60.4 (19.9) years; three females). All participants gave their written informed consent. The experiments conformed to the standards set by the Declaration of Helsinki and were carried out with approval of the local ethics committee.

Eyeblink conditioning

The CS was a loud (~70 dB) 2000 Hz auditory tone lasting 400 ms played via binaural headphones. The CS inconsistently produced an acoustic startle response (''alpha blink'') occurring within 200 ms after the CS.[16] An electrical stimulus (200 μs pulse width at 5× sensory threshold, ~1.0 mA) was given to the supraorbital nerve 400 ms after the CS, to elicit a blink reflex (US).

Repeated pairs of CS and US at 400 ms intervals (ie, delay eyeblink conditioning) yield conditioned blink responses (CRs) occurring within 200 ms before the US.[16] Eyeblink conditioning at this interval is independent of the basal ganglia and cerebral function.[15 17] Examples of conditioned and unconditioned responses are demonstrated in fig 1A.

Conditioning consisted of six learning blocks of 11 trials: trials 1–9 were always CS-US pairs, trial 10 was US only and trial 11 was CS only. The US only trial was for detecting spontaneous blinks and the CS only trial was to confirm that CRs were being acquired, independent of the US. A seventh block consisted of 11 CS only trials to measure extinction. The inter-trial interval was randomised between 10 and 30 s to reduce habituation. To detect any spontaneous blinks, we recorded 400 ms before the CS (800 ms before the US). For patients with dystonia, the unaffected side was conditioned. For normal subjects, the left obicularis oculi (ie, non-dominant side) was conditioned. Subject P11 had a right hearing impairment, but auditory tone volume adjusted for his hearing threshold (50 dB above the auditory threshold, ie, ~90 dB) was sufficient to produce ''alpha blinks''. There were no statistically significant gender or side

differences found (see supplementary data online) and none has been described in the literature.

Electromyographic (EMG) activity was recorded as standard from both obicularis oculi muscles. Blink reflex latency and blink reflex recovery was measured before conditioning bilaterally using a pseudo-random paired pulse technique at 200, 300, 400 and 1000 ms inter-stimulus intervals.

Data and statistical analysis

For measurement of R2 and the blink reflex recovery cycle, EMG data were rectified and averaged over eight trials. R2 latency was measured from the first EMG deflection 30 ms after the stimulus. The blink reflex recovery was represented as conditioned R2 area normalised to unconditioned R2 area.

For measurement of the eyeblink conditioning, the CRs were counted manually. EMG bursts were regarded as "alpha blinks" if their amplitude exceeded 50 μV and if latency was <200 ms after the CS. EMG bursts were regarded as CRs if latency was >200 ms after the CS but before the US. For the CS only trials, EMG bursts occurring 200–600 ms after the CS were considered CRs.

Statistical analysis was performed using SPSS 12.0 (SPSS for Windows, USA). To analyse CRs, two factorial repeated measures ANOVA was used with the within subject factor block (block1, block2, block3, block4, block5, block6) and the between subject factor group (dystonia, normal). For the extinction block, only subjects who were successfully conditioned (>40% CRs in any block) were analysed. Statistical significance was defined as $p<0.05$.

RESULTS

Mean R2 latency was 33.4 (0.7) ms in patients and 34.1 (1.1) ms in controls. Blink reflex excitability was raised in patients with cervical dystonia compared with normal controls (fig 1B) with an effect of group ($F(1,13) = 4.82$, $p = 0.047$). The blink reflex excitability in hand dystonia however was not significantly raised (group effect: $F(1,11) = 0.075$, $p = 0.790$).

Comparing CRs produced per block, patients had a lower rate of CRs as the blocks progressed (fig 1C). This was confirmed by analysis with a two factorial ANOVA of block (within subject factor) and group (between subject factor) showing an interaction of block × group ($F(5,90) = 2.54$, $p = 0.034$). The effect of block was also significant ($F(5,90) = 6.43$, $p<0.001$) but not group ($F(1,18) = 3.45$, $p = 0.080$). Latencies of CRs, spontaneous blink rates and "alpha blinks" were not significantly different between patients and healthy controls (see supplementary table online).

Only five patients and six controls acquired the CR and the extinction phase of these subjects were analysed. Controls exhibited statistically significant extinction (Student's paired t test, $p = 0.013$) but the patients did not (fig 1D). However, a two factorial ANOVA of block6 and block7 showed no significant effect of the group factor ($F(1,9) = 0.83$, $p = 0.39$) or group × block interaction ($F(1,9) = 3.37$, $p = 0.099$). However, this may be due to insufficient statistical power as few patients acquired the responses.

There was no correlation between blink reflex excitability of the conditioned side and the proportion of CRs in patients with dystonia (Spearman's rho = −0.346, $p = 0.270$ for mean of all interstimulus intervals (ISIs); Spearman's rho = −0.311, $p = 0.325$ for 400 ms ISI).

Table 1 Summary of patient characteristics

Patient No	Gender	Age (years)	Dystonia details	Disease duration (years)	Handedness
P1	M	46	R writer's dystonia	2	Right
P2	M	48	R cervical dystonia	8	Right
P3	M	49	L musician's dystonia	12	Right
P4	M	54	R writer's dystonia	20	Right
P5	F	61	R cervical dystonia	10	Right
P6	M	61	L cervical dystonia	40	Left
P7	M	61	R writer's dystonia	6	Right
P8	F	68	R cervical dystonia	10	Right
P9	F	73	L cervical dystonia	17	Right
P10	M	78	R cervical dystonia	6	Right
P11	M	78	R cervical dystonia	25	Right
P12	M	82	R musician's dystonia	15	Right
Mean age		63.3	Mean disease duration	14.3	
SD		12.6	SD	10.4	

DISCUSSION

Blink reflex hyperexcitability in dystonia

The impaired eyeblink conditioning could possibly be due to the R2 blink reflex circuit that is known to be abnormally hyperexcitable in cervical and cranial dystonia but not focal hand dystonia,[18–20] and is also shown in fig 1B. However, patients with focal hand dystonia have normal blink reflex excitability but were similarly impaired in producing CRs (fig 1C). Also, the blink reflex abnormalities and total number of CRs produced were not statistically correlated. Thus the abnormal blink reflex excitability seen in patients with cervical dystonia would not account for the impairment in eyeblink conditioning in this study.

Localisation of the olivo-cerebellar dysfunction in primary focal dystonia

The inferior olive and the deep cerebellar nuclei are well established as being involved in eyeblink conditioning.[7] The role of the cerebellar cortex in this paradigm, however, is still debated. Human studies of cerebellar stroke have suggested that lesions affecting lobule VI of the cerebellar cortex are particularly linked with impairments in eyeblink conditioning.[12 13] Thus the impairment in eyeblink conditioning may be due to either abnormalities in the inferior olive, the cerebellar nuclei or abnormalities in the cerebellar cortex. The finding on structural imaging of grey matter abnormalities in lobules V and VI of the cerebellar cortex of patients with focal dystonia[1 3] is very striking as it coincides spatially with the areas of the cerebellar cortex linked with reduced CRs in eyeblink conditioning.[12 13] Thus it would be parsimonious (although unproven) to suggest that lobule VI of the cerebellar cortex is the source of the impairment found in this study.

A shortcoming might be the inclusion of two forms of adult onset primary focal dystonia, as (in theory) different underlying mechanisms might be involved. However, recent functional imaging studies have indicated altered cerebellar activity in writer's dystonia[7] and blepharospasm,[9] suggesting a shared pathophysiology in the various primary focal dystonia subtypes. This combined with changes in functional activity in the cerebellum of non-manifesting DYT1 carriers[8] suggests a role for the cerebellum in both primary adult onset focal and early onset generalised dystonia.

CONCLUSION

Our study provides neurophysiological evidence for a role of the cerebellum in the pathophysiology of adult onset, primary focal

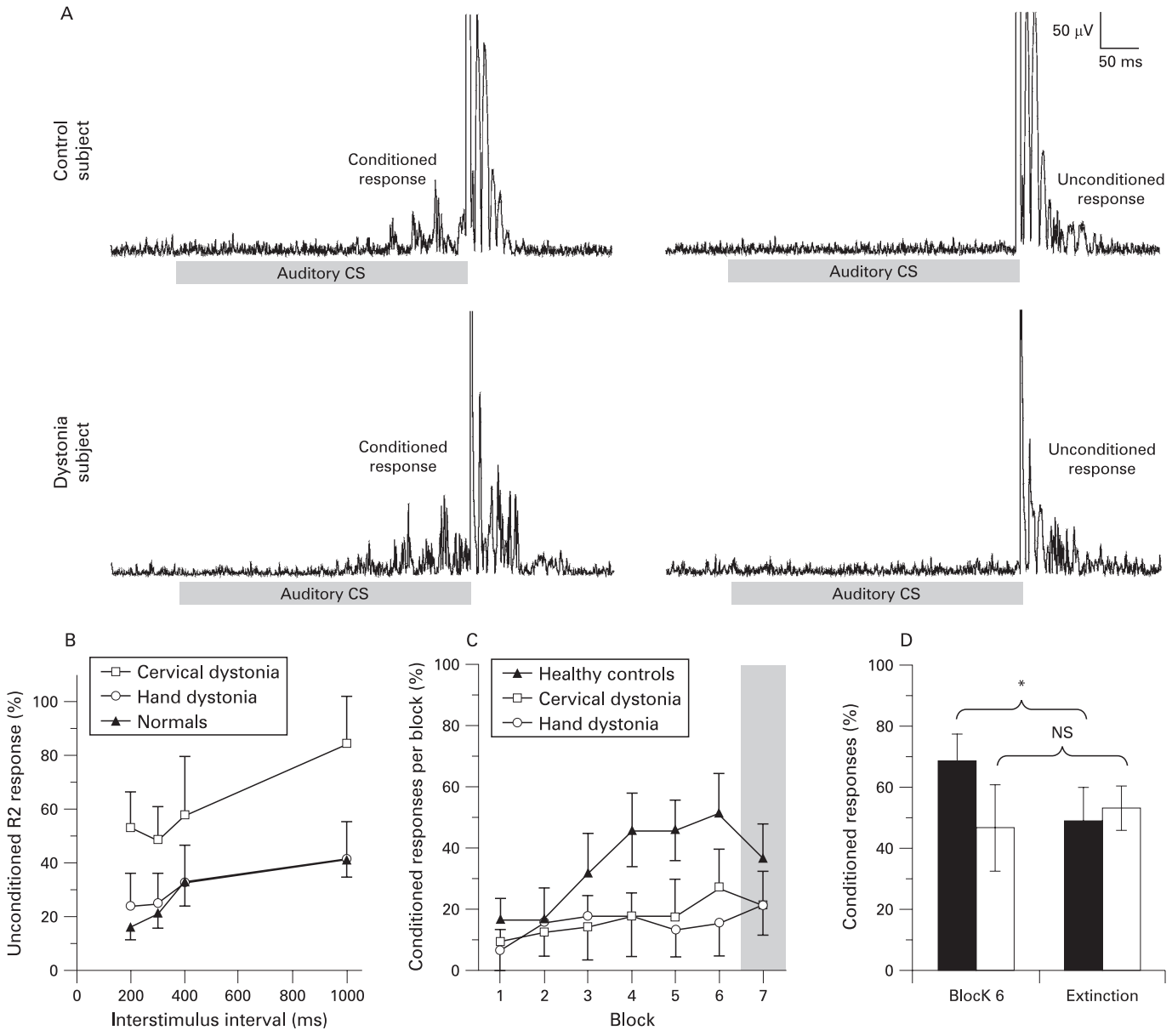

Figure 1 (A) Example of an unconditioned and conditioned response. (B) Blink reflex excitability curve of the conditioned side showing that patients with cervical dystonia had raised blink reflex excitability but not patients with hand dystonia. (C) Patients with adult onset focal dystonia produced less conditioned eyeblink responses compared with normal controls.(D) Extinction phase after eyeblink conditioning in subjects who produced consistent conditioned responses (five patients, six controls). *$p<0.05$. CS, conditioning stimulus.

dystonias. As yet it is unclear whether these functional abnormalities in the cerebellum are causative, secondary or epiphenomenal to the disease, and further work will be needed to establish this.

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