Temporal discrimination threshold and blink reflex recovery cycle in cervical dystonia – two sides of the same coin?

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Title character count: 116

Word count abstract: 229 (< 250)

Word count paper: 1978 (< 2000)

Number of references: 13 (> 12)

Number of figures: 2 (<=2)

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Key words: dystonia, temporal discrimination threshold, blink reflex recovery cycle, endophenotype

Author Disclosures:

Johanna Junker - received a travel grant of the Movement Disorder Society.

Theresa Paulus – reports no disclosures.

Valerie Brandt – received funding from the Strategic Interdisciplinary Research Fund (SIRDF; 07/17-06/18), the Public engagement with Research unit (PERu) and the ROLI music company.

Anne Weissbach - has received a scholarship of the Deutsche Forschungsgesellschaft (DFG; WE5919/1-1) and a Habilitation stipend of the University of Lübeck (H03-2016). She receives funding from the Else Kröner-Fresenius Foundation (2018_A55) and the Deutsche Forschungsgemeinschaft (DFG; WE5919/2-1). She is supported by an Edmond J. Safra fellowship in Movement Disorders from the Michael J Fox foundation.

Sinem Tunc - is supported by the German Research Foundation (SFB 936/project C5). She received travel grants from the Movement Disorders Society and Merz.

Sebastian Loens - is supported by the german federal research ministry (BMBF) through the Dystonia Translational Research and Therapy Consortium (DysTract)) and received a travel grant from abbvie.

Richard Reilly - received research grants from the Health Research Board of Ireland (CSA-2012-5) and the Trinity Centre for Biomedical Engineering and is support by the Dystonia Medical Research Foundation.

Michael Hutchinson - received research grants from Dystonia Ireland, the Health Research Board of Ireland (CSA-2012-5), Foundation for Dystonia Research (Belgium) and the Irish Institute of Clinical Neuroscience.

Tobias Baeumer - was supported by a grant of the Possehl Foundation. His work was also supported by the Deutsche Forschungsgemeinschaft (DFG; SFB 936/project C5) and by the german Bundesministerium für Bildung und Forschung (BMBF) through a project grant (Dystonia Translational Research and Therapy Consortium (DysTract)). He received Honiara from Merz Pharmaceuticals, Allergan and Ipsen Pharma.

Abstract

Introduction: Elevated temporal discrimination thresholds (TDT) have been found in cervical dystonia (CD) and unaffected first-degree relatives, indicating autosomal dominant inheritance with reduced penetrance, serving as an endophenotype and being indicative of abnormal inhibitory processing within the brainstem-basal ganglia circuits. The blink reflex R2 recovery cycle (BRRC) is also a measure of excitability of brainstem-basal ganglia circuits, and inconsistent findings are reported in CD. The aim was to investigate TDT and BRRC in CD and evaluate its reliability as an endophenotype.

Methods: 29 patients with isolated cervical dystonia (mean age: 56.1±14.3, female n=18) and 29 age- and gender-matched healthy controls (mean age: 56.0±14.2, female n=18) were evaluated using a TDT-paradigm, performed as previously described by testing visual, tactile and visual-tactile temporal discrimination thresholds, and the BRRC, investigated with electrical and air puff stimulation.

Results: Mean visual-tactile ($p = .001$) and visual TDTs ($p = .015$) differed between CD and controls; tactile TDTs revealed no group differences ($p = .232$). No between group differences were found for BRRC using either electrical or air puff stimulation ($p= .117$). There was no correlation between the elevation of TDTs and the degree of BRRC-inhibition in CD.

Conclusion: Our findings support the hypothesis that the TDT is an endophenotype in CD. BRRC testing did not demonstrate disinhibition of brainstem-basal ganglia circuits in CD. In contrast to TDT, the BRRC seems not to represent an endophenotype in cervical dystonia.

Highlights:

- Temporal discrimination thresholds are elevated in cervical dystonia
- Blink reflex R2 recovery cycle is not altered in cervical dystonia
- TDT but not BRRC potentially represents an endophenotype in cervical dystonia

Introduction

Elevated temporal discrimination thresholds (TDTs) have been found in patients with dystonia, Parkinson´s disease and multiple system atrophy[1]. Thus basal ganglia dysfunction and defective inhibitory interneural processing within the brainstem (midbrain) - basal ganglia - primary somatosensory cortex - cerebellum circuits are assumed to account for abnormal TDTs in dystonia[1]. Elevated TDTs have been found in CD with high sensitivity (97%) and specificity (98-100%) and also in 50% of female unaffected first-degree relatives, arguing for autosomal dominant heredity with reduced penetrance[2]. The hypothesis of abnormal TDT being an endophenotype of CD, therefore, has been established, suggesting that dystonia with an underlying genetic contribution passes through an endophenotype, which is more penetrant than the phenotype[1].

The blink reflex R2 recovery cycle (BRRC) is a measure of excitability of brainstem-basal ganglia circuits, and controversial findings are reported in CD[3].

This study aimed to investigate and compare excitability of brainstem - basal ganglia - circuits in CD using TDT and BRRC to verify the hypothesis of endophenotypes. Measuring methods of endophenotypes in suspected genetic conditions with low penetrance could help to identify asymptomatic mutation carriers.

Methods:

Participants:

TDT and BRRC were determined in 30 patients with isolated CD and 30 age- and gender-matched healthy controls. BRRC data of four healthy controls from a previous study were included[4]. Exclusion criteria were secondary dystonia, an affection of peripheral nerves or CNS, DBS, medication affecting the CNS and contact lenses. Dystonia severity was evaluated

using the Burke-Fahn-Marsden Dystonia Rating Scale. Patients receiving botulinum toxin were enrolled with an interval of at least three months after the last injection. All participants gave written informed consent for study participation prior to study enrollment. The local ethics committee approved the study.

Temporal Discrimination Threshold

The TDT paradigm was performed as previously described by testing visual, tactile, and visual-tactile temporal discrimination thresholds bilaterally[2]. Sensory perception threshold was detected by increasing stimulus intensities using square-wave stimulators (DS7A Digitimer; Digitimer Limited, Welwyn Garden City, U.K.; 0.1mA steps; pulse length 0.5ms, 400V). Participants received pairs of non-painful electrical stimuli (twice of sensory perception threshold) on the index and middle finger, pairs of LED flashlights, positioned seven degrees into the peripheral visual field, or the combination of a visual and tactile stimulus on the index finger with ascending interstimulus intervals (ISI). Pairs of stimuli were first presented synchronously, then ISIs increased in 5ms steps. The interval between pairs of stimuli was 5sec. Each of the three modalities (visual, tactile, visual-tactile) was tested four times per body side. Reporting of asynchronous perception of stimuli on three consecutive occasions, the first accounted as the threshold. The mean values of the two median thresholds per body side represent visual, tactile and visual-tactile TDT.

Blink Reflex R2-Recovery Cycle

The BRRC was investigated with electrical and air puff stimulation using previously described paradigms[4]. The blink reflex was elicited by pairs of electrical stimuli to the right supraorbital nerve or by pairs of air puffs to the lateral canthus of the right eye with different ISIs (200,

300, 500, 1000, 3000ms) in a pseudorandomized order. Intertrial interval was 30sec, supramaximal electrical stimulation was always followed by an eyeblink, and stimulus pulse length was 0.2ms for electrical stimulation and 100ms for air puff stimulation. The EMG of the orbicularis oculi muscle was recorded using Ag-AgCl surface electrodes. The EMG signals were amplified, filtered (high-pass filter 20 Hz, low-pass filter 1 kHz, 1902 amplifier and Micro 1401 laboratory interface; both Cambridge Electronic Design Ltd, Cambridge, UK) and stored on a PC. The area under the curve of unconditioned and conditioned R2 was calculated. Means of 6 trials were calculated for unconditioned and conditioned R2, and the R2 recovery index of each participant was the mean percentage of conditioned R2 relative to unconditioned R2.

Statistical Analysis

Statistical analyses for TDT and BRRC were performed using repeated-measure ANOVAs and post-hoc t-Test where interactions became significant. The Greenhouse-Geisser method was used to correct for non-sphericity.

To evaluate the relation between elevated TDTs (visual, tactile, visual-tactile) and suppression of conditioned R2, Spearman`s rank correlations were performed for patients and controls respectively.

All tests of significance were two-sided. P-values ≤ .05 were considered significant, p-values for multiple comparisons were Bonferroni-corrected. Analyses were run in SPSS 22.

Results:

Participants:

One CD patient had to be excluded due to secondary dystonia. Mean BFMDRS of the remaining 29 patients with isolated CD (mean age: 56.1 ± 14.3 years, female n = 18) was 4.6 ± 1.8. 29 age- and gender-matched healthy controls (mean age: 56.0 ± 14.2 years, female n = 18) were included.

Temporal discrimination thresholds:

A repeated measure ANOVA with *type of stimulation* [visual, tactile, visual-tactile] as a within-subjects factor and *group* as a between-subjects factor revealed main effects for *type of stimulation* [$F(1.52, 85.26) = 57.1$, $p < 0.001$, $\eta^2 = 0.51$], *group* [$F(1,56) = 10.81$, $p = 0.002$, $\eta^2 = 0.16$] and the interaction between *type of stimulation* and *group* [$F(1.52, 85.26) = 9.16$, $p = 0.001$, $\eta^2 = 0.14$]. Post-hoc t-tests showed *group* differences for visual-tactile TDTs (CD 140.9 ± 60.41ms vs. controls 86.8 ± 46.95ms, $t(28) = 3.57$, $p = 0.001$) and visual TDTs (CD 70.35 ± 21.87ms vs. controls 54.78 ± 26.39ms, $t(28) = 2.58$, $p = 0.015$), but not for tactile TDTs (CD 69.44 ± 33.35 vs. controls 57.76 ± 35.64, $t(28) = 1.22$, $p = 0.232$) as shown in figure 1. Post-hoc t-tests between *types of stimulation* showed differences between visual and visual-tactile TDTs (CD: $t(28) = -7.62$, $p < 0.001$, controls: $t(28) = -3.95$, $p < 0.001$) and between tactile and visual-tactile TDTs (CD: $t(28) = -8.67$ $p < 0.001$, controls: $t(28) = -3.08$, $p = 0.005$), indicating significant higher TDT values of the visual-tactile modality. No significant differences were found between visual and tactile TDTs (CD: $t(28) = 0.18$, $p = 0.856$, controls: $t(28) = -0.56$, $p = 0.583$).

Blink reflex recovery cycle:

A repeated measures ANOVA with *type of stimulation* [air puff stimulation, electrical stimulation] and *ISI* as within-subjects factors and *group* as a between-subjects factor revealed a main effect for *type of stimulation* [$F(1, 56) = 6.05$, $p = 0.017$, $\eta^2 = 0.10$], *ISI* [$F(3.32,185.87) = 171.32$, $p < 0.001$, $\eta^2 = 0.75$] and the interaction between *type of stimulation* and *ISI* [$F(3.2,179.33) = 11.14$, $p < 0.001$, $\eta^2 = 0.17$]. No significant *group* differences [$F(1,56) = 2.34$, $p = 0.132$, $\eta^2 = 0.04$] and no significant interactions were found between *type of stimulation* and *group* [$F(1,56) = 0.28$, $p = 0.602$, $\eta^2 = 0.01$], between *ISI* and *group* [$F(3.32,185.87) = 0.78$, $p = 0.516$, $\eta^2 = 0.01$], between *type of stimulation*, *ISI* and *group* [$F(3.2,179.33) = 0.84$, $p = 0.480$, $\eta^2 = 0.02$]. Post hoc t-tests revealed differences between *types of stimulation* for ISI 200ms ($t(57) = -4.08$, $p < .001$), ISI 300ms ($t(57) = -3.05$, $p = 0.003$) and ISI 3000ms ($t(57) = 3.22$, $p = 0.002$), indicating less suppression (ISI 200ms and 300ms) and less recovery (ISI 3000ms) of conditioned R2 in participants using air puff stimulation (figure 2). No differences were found for ISI 500ms ($t(57) = -1.97$, $p = 0.054$) and ISI 1000ms ($t(57) = -1.85$, $p = 0.069$).

Relation between temporal discrimination thresholds and blink reflex recovery cycle:

Evaluation of relation between elevated TDTs (visual, tactile, visual-tactile) and suppression of conditioned R2 (BR-ratio at ISI 200ms for electrical and air stimulation) in patients revealed no correlation between any TDT and BR value (all $r \leq 0.27$, all $p \geq 0.153$). No correlation between blink reflex suppression and TDTs was found for controls (all $r \leq 0.16$, all $p \geq 0.273$).

Discussion

This is the first study evaluating the relationship between TDT and BRRC in CD. We confirmed the previous finding of elevated TDTs in CD, but found no abnormalities of BRRC in CD, indicating that BRRC presumably does not represent an endophenotype in CD.

TDT is known as a quantitative measure of the ability to discriminate or perceive rapid changes in the environment, and abnormal TDTs are likely caused by impaired somatosensory processing in the time domain[1]. Our results of elevated visual and visual-tactile TDTs support the hypothesis of TDT accounting as an endophenotype[1]. Furthermore, our results are in line with other studies, that did not find group differences for tactile TDT in CD[5,6], indicating that tactile TDT is not as sensitive as visual-tactile TDT. The existence of controversial study results concerning TDT modalities could be partly due to methodical differences, the number of patients, and the way of data analyses, as reporting of combined TDTs, mean TDT on group level or Z scores with percentages of altered TDTs within patient groups[2,5-7].

Abnormal TDTs in CD are likely due to defective inhibitory interneural processing within the brainstem (midbrain) - basal ganglia - primary somatosensory cortex – cerebellum - circuit[1]. The superior colliculus as part of the midbrain is considered to be dysfunctional in dystonia[1]. Elevated visual TDTs in CD support the assumption of alterations of the superficial layer of the superior colliculus[8], receiving direct input from the visual system[9]. The pronounced group difference for multimodal TDT (figure 1) indicates dysfunction of the deep layer of the superior colliculus since cat models displayed enhanced neuron responses after multimodal compared to unimodal sensory stimuli[8]. Our results also support the findings of impaired spatial sensory processing in CD, since visual-tactile TDTs may at least partially represent

spatial sensory integration[5]. As the superior colliculus is considered to be a key node for the discrimination of rapid changes in the environment and is also involved in the processing of visual stimuli[1], it may explain the pronounced alterations of the visual and visual-tactile compared to tactile domains. Furthermore, the non-significant differences in the tactile domain might also be explained by the relatively small sample size.

Studies present controversial results of BRRC in CD, some indicating decreased inhibition of the BRRC compared to healthy controls[3], some reporting disinhibition in only 37% of CD[10], while other studies do not reveal any group differences[11]. The controversy of study results may partly be due to methodical differences, which corroborates the importance of homogeneous patient groups, standardized study protocols and assessment across different laboratories. Elevated TDTs but no BRRC abnormalities were found in patients with a prodromal form of blepharospasm, were increased blinking was reported[12]. TDTs remained unchanged and BRRC became abnormal when developing orbicularis oculi muscle spasms later in the course of the disease[12]. As BRRC measures excitability of pontine/medulla oblongata - basal ganglia - circuits[4], caudal brainstem areas may not play a central role in the pathophysiology of CD. These findings may furthermore explain inconsistency across study results, especially relating to BRRC. Hence, while TDT seems to represent a reliable endophenotype of CD, disinhibition of BRRC may be a secondary phenomenon of central disinhibition respectively a phenomenon related to an increase of symptoms throughout the disease. Enhancement of BRRC in eight manifesting and five non-manifesting DYT1 mutation carriers still argues for an endophenotype[13], while normal BRRC in 17 myoclonus patients[4] and the findings of our study questions this hypothesis. To clarify the controversy of study results in CD further investigations in larger groups of genetically defined forms of dystonia as well as other types of dystonia and the correlation with clinical parameters are needed.

This study has certain limitations: Sample sizes are not large enough to detect small effects. However, the groups were well defined and closely matched and the paradigms used are known to elicit robust, large effect sizes. We used the staircase method for the evaluation of TDT to keep comparability with other studies. However TDT being a psycho-physiological task, future studies should investigate the effect of delivering stimuli in a randomized or adaptive order.

Conclusion:

Revealing group differences for TDT supports the hypothesis of TDT accounting as an endophenotype in CD. Disinhibition of brainstem - basal ganglia - circuits in patients with CD could not be detected by BRRC. In contrast to the TDT, the BRRC presumably does not represent an endophenotype in CD.

Acknowledgement:

We would like to thank Elisa Werner, MD, for contributing BRRC data of four controls from a previous study[4].

Figure

Figure 1. Tactile, visual and visual-tactile TDTs in ms in patients with CD and controls. Visual and visual-tactile TDTs were significantly higher in cervical dystonia compared to controls. Tactile TDTs revealed no significant differences between patients and controls. *$p \leq 0.017$ (Bonferroni-corrected); ***$p \leq 0.001$.

Figure 2. R2 ratio in % (conditioned R2/ unconditioned R2) for air (a) and electric stimulation (b) in patients with CD and controls. BRRC revealed no significant differences between patients and controls.

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