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Cerebellar transcranial direct current stimulation modulates timing but not acquisition of conditioned eyeblink responses in SCA3 patients

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ABSTRACT

Background: Delay eyeblink conditioning is an extensively studied motor learning paradigm that critically depends on the integrity of the cerebellum. In healthy individuals, modulation of cerebellar excitability using transcranial direct current stimulation (tDCS) has been reported to alter the acquisition and/or timing of conditioned eyeblink responses (CRs). It remains unknown whether such effects can also be elicited in patients with cerebellar disorders.

Objective: To investigate if repeated sessions of cerebellar tDCS modify acquisition and/or timing of CRs in patients with spinocerebellar ataxia type 3 (SCA3) and to evaluate possible associations between disease severity measures and eyeblink conditioning parameters.

Methods: Delay eyeblink conditioning was examined in 20 mildly to moderately affected individuals with SCA3 and 31 healthy controls. After the baseline assessment, patients were randomly assigned to receive ten sessions of cerebellar anodal tDCS or sham tDCS (i.e., five days per week for two consecutive weeks). Patients and investigators were blinded to treatment allocation. The same eyeblink conditioning protocol was administered directly after the last tDCS session. The Scale for the Assessment and Rating of Ataxia (SARA), cerebellar cognitive affective syndrome scale (CCAS-S), and disease duration were used as clinical measures of disease severity.

Results: At baseline, SCA3 patients exhibited significantly fewer CRs than healthy controls. Acquisition was inversely associated with the number of failed CCAS-S test items but not with SARA score. Onset and peak latencies of CRs were longer in SCA3 patients and correlated with disease duration. Repeated sessions of cerebellar anodal tDCS did not affect CR acquisition, but had a significant treatment effect on both timing parameters. While a shift of CRs toward the conditioned stimulus was observed in the sham group (i.e., timing became more similar to that of healthy controls, presumably reflecting the effect of a second eyeblink conditioning session), anodal tDCS induced a shift of CRs in the opposite direction (i.e., toward the unconditioned stimulus).

Conclusion: Our findings provide evidence that cerebellar tDCS is capable of modifying cerebellar function in SCA3 patients. Future studies should assess whether this intervention similarly modulates temporal processing in other degenerative ataxias.

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1. Introduction

The ability to establish associations between different stimuli and motor behaviours is a fundamental property of the central nervous system. One of the most extensively studied associative learning paradigms in both animal and human literature is delay eyeblink conditioning, which relies on the integrity of a conserved neural circuit comprising specific cerebellar and brainstem structures [1,2]. In brief, repeated pairing of a neutral conditioned stimulus (CS), such as a tone or flash of light, with an aversive unconditioned stimulus (US), such as a weak periorbital shock or corneal air puff, ultimately results in the generation of a well-timed conditioned response (CR), i.e., eyelid closure, prior to presentation of the US. In addition to evaluating an individual's ability to acquire conditioned eyeblink responses, this paradigm lends itself particularly well to investigate cerebellar time processing within the millisecond range in health and disease [3–5].

Transient modulation of cerebellar cortical excitability by repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS) has been shown to affect eyeblink conditioning performance in healthy adult volunteers [4–7]. Polarity-dependent effects of cerebellar tDCS were initially reported by Zuchowski and colleagues who demonstrated enhanced acquisition following anodal tDCS and impaired acquisition following cathodal tDCS compared to sham stimulation [7]. Moreover, anodal tDCS was found to induce a shortening of CR onset and peak latencies (i.e., a shift toward CS onset), while both timing parameters remained unchanged after cathodal tDCS. However, subsequent sham-controlled studies from the same group and others could not replicate these findings, but revealed a delayed appearance of CRs (i.e., a shift toward US onset) after cerebellar anodal and cathodal tDCS [4,5]. Whether such modulating effects on CR timing and/or acquisition also occur in patients with cerebellar disorders has not been previously examined.

Spinocerebellar ataxia type 3 (SCA3) is the most common dominantly inherited degenerative ataxia worldwide. Like most polyglutamine SCAs, neuronal loss in SCA3 is not confined to the cerebellar cortex and deep cerebellar nuclei, but frequently also involves the spinal cord, basal ganglia, brainstem, and peripheral nerves to varying degrees [8]. As a result of these widespread degenerative changes, patients develop progressive motor and cognitive deficits, which significantly affect quality of life and mood [9–14]. Disease-modifying therapies are currently not available and symptomatic treatment options are restricted to physical therapy and rehabilitation programs [15]. In parallel with the increasing scientific application of cerebellar tDCS in healthy individuals, recent years have witnessed an upsurge of interest in this technique as a potential therapeutic strategy in patients with ataxia [16–21]. Of note, repeated sessions might generate cumulative changes in synaptic efficacy, as evidenced by a return of cerebellar brain inhibition and reduction in ataxia severity lasting several months [16,17,20]. However, effects of multiple sessions of cerebellar tDCS on motor learning have not been studied in individuals with ataxia.

In the present study, we aimed to investigate if repeated administration of cerebellar anodal tDCS enhances CR acquisition and/or modulates CR timing in SCA3 patients compared to sham tDCS. In addition, we compared eyeblink conditioning performance between SCA3 patients and healthy controls and examined whether motor and cognitive deficits in SCA3, which are thought to be subserved by distinct cerebellar subregions, are differentially associated with CR acquisition and timing. Finally, in light of the gradually progressive course of SCA3, we also evaluated if CR acquisition and timing are related to disease duration.

2. Methods

2.1. Study design and participants

We conducted a randomized, double-blind, sham-controlled trial at the Radboud University Medical Center in which SCA3 patients were randomly assigned in a 1:1 ratio to receive ten sessions of cerebellar anodal or sham tDCS (i.e., five days per week for two consecutive weeks). Randomization was stratified by Scale for the Assessment and Rating of Ataxia (SARA) score using Castor EDC software, with permuted block sizes of two or four (https://castoredc.com). Delay eyeblink conditioning was performed at baseline and repeated directly after the last tDCS session to evaluate possible changes in CR acquisition and timing. Furthermore, baseline eyeblink conditioning parameters of SCA3 patients were compared with previously collected data by our group from unrelated controls of comparable age and sex without a history of neurological or psychiatric disorders [22–24]. The effects of cerebellar tDCS on ataxia severity and other clinical outcome measures are described in a separate paper [25].

Patients were eligible when they had a confirmed pathogenic trinucleotide repeat expansion in the *ATXN3* gene and mild to moderate ataxia, as defined by a SARA score between 3 and 20 [19,26]. Exclusion criteria were related to tDCS application and included epilepsy, a history of brain surgery, metallic implants in or near the skull, a pacemaker, pregnancy, and a severe skin condition affecting the site of electrode placement. None of the participants reported hearing deficits.

The study was approved by the local ethics committee (CMO region Arnhem-Nijmegen) and registered in the Netherlands Trial Register (NL7321) on October 8, 2018. Written informed consent was obtained from all subjects.

2.2. Eyeblink conditioning

Participants were comfortably seated in a chair with adjustable armrests. Two pairs of Kendall H59P electrodes were used to record surface electromyographic (EMG) activity from both orbicularis oculi muscles. The active and resting electrodes were positioned just caudal to the lower eyelids and 3 cm lateral from the outer canthi, respectively.

The CS consisted of a neutral tone (80 dB, 2 kHz, 400 ms duration) that was presented through binaural headphones. In paired trials, the CS was followed by a coterminating supraorbital electrical stimulus (US, 200 μs pulse width) generated by a Digitimer DS7A (Digitimer Ltd). The cathode was placed over the right supraorbital foramen and the anode was placed 2 cm above the cathode. Stimulus intensities were determined on an individual basis by multiplying the participant's sensory electrical threshold by a factor 7 to 10 [6,24] and were kept similar across both sessions.

The experimental paradigm was composed of six learning blocks and a final extinction block, each containing eleven trials [6,22–24]. In the first nine trials of acquisition blocks, conditioned and unconditioned stimuli were presented in pairs as described above, while in the tenth and eleventh trial only one of them was delivered (trial 10: CS-only, trial 11: US-only). Finally, the extinction block comprised eleven CS-only trials. Beforehand, participants were informed about the presentation of tones and supraorbital electrical stimuli, either alone or in combination, but not about the specific purpose of the investigation and the expected behavioural responses.

EMG recordings ipsilateral to the US were analyzed on a trial-by-trial basis. Similar to previous studies, trials with spontaneous blinks occurring before CS onset were excluded from the analysis [3,7,27]. An EMG burst was classified as a conditioned response

when the following two criteria were met: 1) an onset in the time window between 150 ms after CS onset and US administration in case of a paired CS-US trial *or* between 150 and 600 ms after CS onset in case of a CS-only trial and 2) a duration of at least 50 ms or a transition into a superimposed unconditioned response (UR) [3,24,27]. Onset and peak times of CRs were visually identified and expressed in milliseconds prior to US presentation (using negative values). The former was defined as the earliest point at which EMG activity began to rise significantly from the pre-CS baseline level [3,6]. If the CR consisted of multiple peaks, the latency to the peak with the largest amplitude was taken. EMG activity within the first 150 ms following a CS was considered to be an acoustic startle response, also known as alpha blink. Finally, UR onset and peak latencies in US-only trials were determined. The investigator who performed the data analysis was blinded to the randomization status of SCA3 patients.

2.3. Clinical evaluation of disease severity

Ataxia severity in SCA3 patients was quantified using the SARA score, which ranges from 0 (no ataxia) to 40 (most severe ataxia) [26]. In addition, the cerebellar cognitive affective/Schmahmann syndrome scale (CCAS-S) was administered to assess neuropsychological deficits. Outcomes of this screening instrument, which includes semantic fluency, phonemic fluency, category switching, digit span forward, digit span backward, cube drawing, delayed verbal recall, similarities, go/no-go, and affect regulation items, are expressed as a number of failed tests ranging from 0 to 10 and a total score ranging from 0 to 120 points [28]. Finally, as a more global measure of disease severity, disease duration was calculated by subtracting the age of onset of gait ataxia from the patient's age at baseline.

2.4. Transcranial direct current stimulation

Cerebellar tDCS was delivered through two rubber electrodes (7 cm × 5 cm, current density: 0.057 mA/cm^2) that were connected to a neuroConn DC stimulator (neuroConn GmbH, Ilmenau, Germany). The anode was covered in a saline-soaked sponge and placed horizontally over the midline 2 cm below the inion, while the cathode was positioned over the right deltoid muscle. Patients received ten sessions of anodal tDCS or sham tDCS, which shared a similar ramp-up and ramp-down period of 30 s each. A constant current of 2 mA was administered for 20 min in the anodal tDCS condition, while each sham session, by convention, contained 40 s of real stimulation and 1160 s of continuous impedance control without stimulation. The study mode of the neuroConn stimulation device was used to ensure that tDCS application occurred in a double-blind fashion. The success of blinding was assessed at the end of the two-week intervention period when participants were asked about their impression of treatment allocation.

2.5. Statistical analysis

The primary objective of this randomized controlled study was to examine whether repeated sessions of cerebellar tDCS induce a decrease in ataxia severity in individuals with SCA3 [25]. Changes in eyeblink conditioning parameters were secondary endpoints. Power calculations were based on the mean reduction in SARA score reported by a previous trial with an identical tDCS protocol (estimated effect size *f* of 0.92) [17]. We determined that a sample size of twenty participants with five measurements each would yield a power of 0.99 to detect significant differences when SARA score was chosen as the primary outcome measure.

The incidence of CRs per block, overall incidence of CRs across the six learning blocks, onset and peak latencies of CRs in paired trials, number of alpha blinks, and peak latency of URs were compared between SCA3 patients and healthy controls using Mann-Whitney *U* tests or independent samples *t*-tests. As visual inspection of histograms and Shapiro-Wilk tests revealed that the percentage of CRs over different blocks in both SCA3 patients and healthy controls was not normally distributed, non-parametric tests were used to analyze these data. Differences in CR incidence between blocks 6 and 7 of the first session (extinction) and between block 6 of the first session and block 1 of the second session (retention) were evaluated by Wilcoxon signed-rank tests. Associations between clinical disease severity measures and eyeblink conditioning performance in SCA3 patients were explored using Spearman's rank-order correlation coefficients or Pearson's correlation coefficients, depending on the distribution of data. In order to determine possible treatment effects of cerebellar tDCS on CR acquisition and timing, linear regression analyses were employed with overall CR incidence, onset latency, and peak latency after two weeks as outcome variables, the respective baseline scores as covariate, and stimulation group as categorical variable. Finally, Spearman's rank-order correlation coefficients were used to assess possible associations between changes in eyeblink conditioning parameters (i.e., overall incidence of CRs as well as onset and peak latencies) and baseline demographic and clinical characteristics (i.e., age, disease duration, SARA score, CCAS-S score, and number of failed CCAS-S test items) in patients who had received anodal tDCS. Statistical analyses were performed in SPSS Statistics (IBM, version 25). The level of significance was set at $p < 0.05$ (two-sided).

3. Results

3.1. Demographic and clinical characteristics of participants

Twenty SCA3 patients (12 males; mean age ± SD, 51.9 ± 10.0 years) and 31 healthy controls (13 males; mean age ± SD, 47.9 ± 16.5 years) were included. Patients had a mean disease duration of 8.0 years (SD 5.4 years), SARA score of 11.9 points (SD 3.9 points), and CAG repeat length of 67.6 (SD 3.4). No relevant differences were observed between SCA3 patients in both stimulation groups regarding age (anodal tDCS: 52.4 ± 10.8 years; sham tDCS: 51.4 ± 9.8 years), sex (anodal tDCS: 7 males; sham tDCS: 5 males), age of onset (anodal tDCS: 45.2 ± 9.9 years; sham tDCS: 42.6 ± 8.8 years), disease duration (anodal tDCS: 7.2 ± 4.7 years; sham tDCS: 8.8 ± 6.2 years), SARA score (anodal tDCS: 11.3 ± 3.2 points; sham tDCS: 12.5 ± 4.7 points), CCAS-S score (anodal tDCS: 80.3 ± 7.0 points; sham tDCS: 83.4 ± 11.2 points), and number of failed CCAS-S test items (anodal tDCS: 3.2 ± 1.2; sham tDCS: 2.9 ± 2.3).

3.2. Eyeblink conditioning performance in SCA3 patients versus healthy controls

Mean percentages of CRs per block, the overall percentage of CRs in blocks 1 to 6, and standard errors in SCA3 patients and healthy controls are shown in Fig. 1A. A significant increase in the number of CRs across the six learning blocks was observed in healthy controls ($\chi^2(5) = 55.16$, $p < 0.001$) and, to a lesser extent, also in SCA3 patients ($\chi^2(5) = 23.47$, $p < 0.001$). Mann-Whitney *U* tests revealed significant differences between both groups in the overall percentage of CRs ($U = 112.0, z = -3.84, p < 0.001$) as well as in the percentage of CRs in each of the learning blocks (all *p* values ≤ 0.005). Eleven out of twenty patients exhibited at least one CR in the sixth learning block, while the ability to acquire CRs in this baseline session was found to be completely abolished in the other

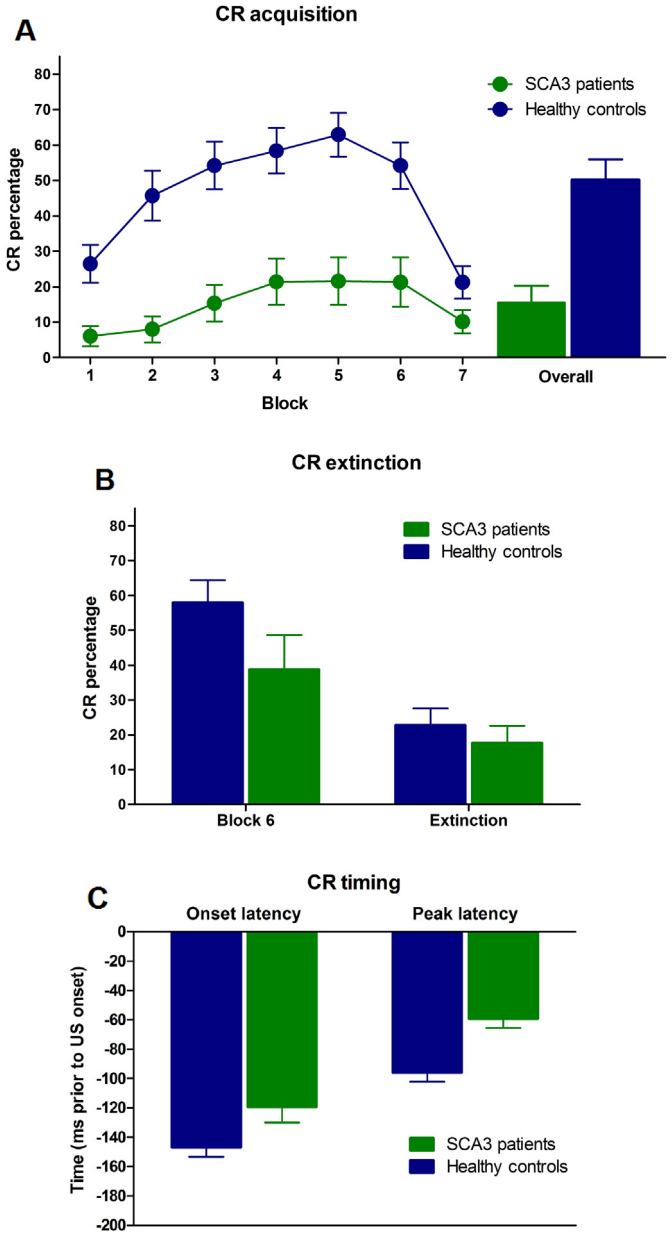

Fig. 1. Acquisition (A), extinction (B), and timing (C) of conditioned eyeblink responses (CRs) in mildly to moderately affected patients with spinocerebellar ataxia type 3 (SCA3) and healthy controls.
A. Mean percentages of CRs and standard errors are shown per block in SCA3 patients (green circles) and healthy controls (blue circles). The two bars on the right indicate the overall percentage of CRs in blocks 1 to 6. Compared with healthy controls, SCA3 patients exhibited significantly fewer CRs in each of the six learning blocks (all p values ≤ 0.005) as well as in blocks 1 to 6 together ($p < 0.001$). B. Mean percentages of CRs and standard errors in blocks 6 and 7 (extinction) in SCA3 patients and healthy controls. As the extinction analysis only involved individuals with at least one CR in block 6, values are slightly different from those in panel A. Differences between both blocks were significant in healthy controls ($p < 0.001$), indicating successful extinction, but not in SCA3 patients ($p = 0.075$). C. Means and standard errors of onset and peak latencies of conditioned eyeblink responses in paired trials in SCA3 patients and healthy controls. Note that negative values indicate time before presentation of the unconditioned stimulus (US). Compared with healthy controls, SCA3 patients had significantly longer onset latencies ($p = 0.037$) and peak latencies ($p = 0.001$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

nine. A comparison between these two groups showed that the latter individuals had significantly lower CCAS-S scores (77.1 ± 9.1 versus 85.7 ± 7.7 points; $p = 0.034$) and a higher number of failed CCAS-S test items (4.1 ± 2.0 versus 2.2 ± 1.2; $p = 0.018$). There were no significant between-group differences in SARA score (13.1 ± 4.0 versus 10.9 ± 3.8 points; $p = 0.22$), disease duration (9.4 ± 6.7 versus 6.8 ± 4.1 years; $p = 0.40$), and level of disability in conducting activities of daily living, as measured by part II of the Friedreich Ataxia Rating Scale (13.6 ± 3.9 versus 11.2 ± 3.3 points; $p = 0.16$).

Twenty-nine healthy controls and eleven SCA3 patients exhibited at least one CR in block 6 and were included in the analysis of extinction (Fig. 1B). Compared to the last block with paired trials, a significant decline in the percentage of CRs occurred in the extinction block in healthy controls ($Z = -3.94$, $p < 0.001$) with only a trend in SCA3 patients ($Z = -1.78$, $p = 0.075$).

As shown in Fig. 1C, onset and peak latencies of CRs in paired trials were longer (i.e., closer to the US) in individuals with SCA3 than in healthy controls (onset latency: $t = -2.16$, $p = 0.037$; peak latency: $t = -3.47$, $p = 0.001$). In order to exclude pathology in the response output circuitry as a possible cause of this delayed occurrence of CRs, timing of URs was also compared between groups but found to be not significantly different ($t = -1.25$, $p = 0.22$). Finally, SCA3 patients displayed fewer alpha blinks than healthy controls ($Z = -5.35$, $p < 0.001$).

3.3. Associations between eyeblink conditioning performance and disease severity measures in SCA3 patients

The overall percentage of conditioned eyeblink responses in blocks 1 to 6 was inversely associated with the number of failed CCAS-S test items ($\rho = -0.58$, $p = 0.008$) but not with SARA score ($\rho = -0.31$, $p = 0.19$) or disease duration ($\rho = -0.26$, $p = 0.26$). Mean onset latency of CRs, on the other hand, was correlated with disease duration ($\rho = 0.73$, $p = 0.011$), as shown in Fig. 2, but not with the number of CCAS-S failures ($\rho = -0.08$, $p = 0.82$) or SARA score ($r = 0.27$, $p = 0.43$). Similar relationships were found between CR peak latency and disease duration ($\rho = 0.67$, $p = 0.024$), SARA score ($r = 0.44$, $p = 0.18$), and the number of failed CCAS-tests ($\rho = -0.10$, $p = 0.77$). Correlations between both timing parameters and disease duration indicate a shift of CRs closer to US onset in SCA3 patients as time since disease onset increases. Conversely, associations between disease duration and UR onset and peak latency were not significant ($p > 0.10$).

3.4. Effects of cerebellar anodal tDCS on eyeblink conditioning performance in SCA3 patients

As an index of retention, we compared the percentage of CRs between block 6 of the baseline session and block 1 of the follow-up session, thereby only including individuals with at least one CR in block 6 of the first session. In line with previous studies in degenerative cerebellar diseases, we found a significant difference between these blocks in SCA3 patients ($Z = -2.5$, $p = 0.012$) reflecting impaired retention [27,29]. Similar results were obtained when only patients in the sham group were included in the analysis ($Z = -2.4$, $p = 0.018$).

Although there was some increase in the percentage of CRs in both treatment arms in the second session, especially over the course of the first three blocks (Fig. 3), Friedman tests did not reveal a significant learning effect (anodal tDCS: $\chi^2(5) = 5.69$, $p = 0.34$; sham tDCS: $\chi^2(5) = 10.50$, $p = 0.062$). After adjusting for baseline performance, the overall percentage of CRs after two weeks of tDCS was not affected by stimulation condition ($b = 3.9$, 95% confidence interval [CI] −5.6 to 13.4, $p = 0.40$).

At two-week follow-up, there were significant between-group differences in CR onset latency ($b = 72.1$, 95% CI 41.9 to 102.3, $p = 0.001$) and CR peak latency ($b = 29.3$, 95% CI 5.8 to 52.8,

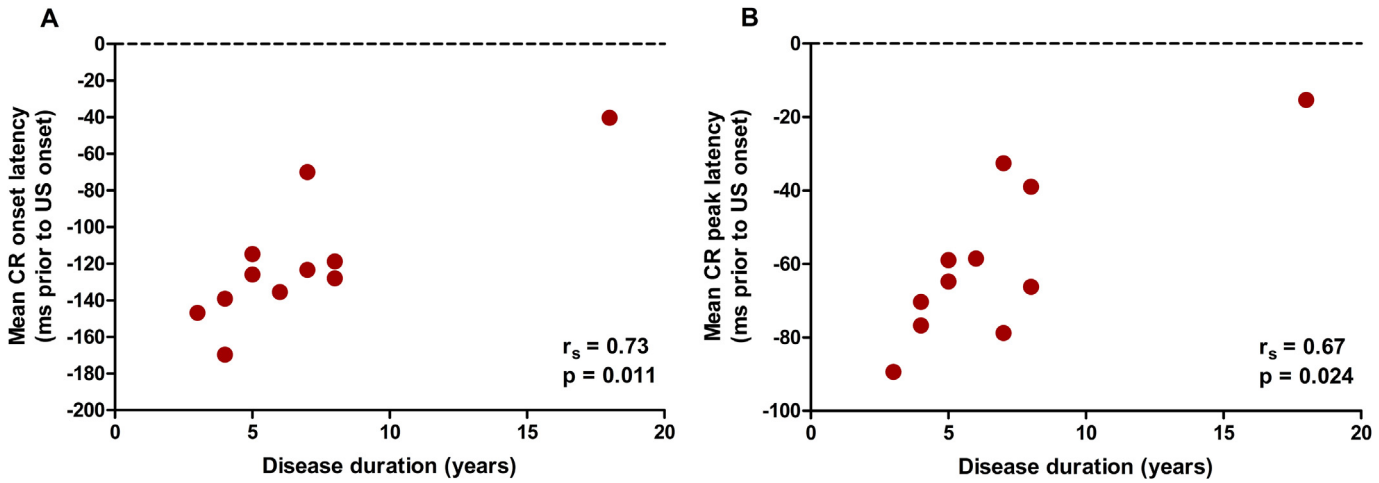

Fig. 2. Associations between disease duration and timing of conditioned eyeblink responses (CRs) in mildly to moderately affected patients with spinocerebellar ataxia type 3. As disease duration increased, a shift of CR onset (A) and peak time (B) was noticed in the direction of the unconditioned stimulus (US). Note that negative values indicate time before US presentation.

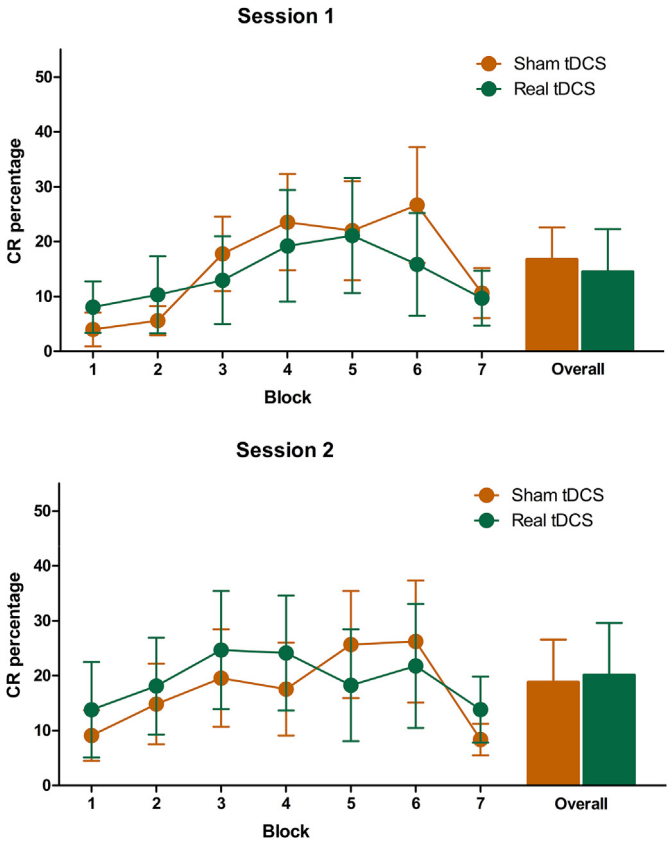

Fig. 3. Acquisition of conditioned eyeblink responses (CRs) at baseline on the left and after ten sessions of cerebellar anodal tDCS (green circles) or sham tDCS (orange circles) on the right in mildly to moderately affected patients with spinocerebellar ataxia type 3. Shown are mean percentages of CRs and standard errors per block as well as the overall percentage of CRs in blocks 1 to 6. Acquisition of CRs was not significantly modulated by cerebellar anodal tDCS. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

$p = 0.021$) after adjustment for baseline values. Compared with the baseline measurement, average CR onset latency increased in SCA3 patients who had received ten sessions of anodal tDCS (i.e., a shift in the direction of the US), while an opposite pattern was observed in the sham arm (i.e., a shift in the direction of the CS) (Fig. 4A). In parallel with the change in onset latency, average CR peak time occurred closer to the US after two weeks of anodal tDCS (Fig. 4B). Peak latency slightly decreased in patients in the sham group. In sum, these data indicate a treatment effect of cerebellar anodal tDCS on both CR onset and peak latency but not acquisition.

No significant associations were found between changes in eyeblink conditioning parameters and baseline demographic and clinical characteristics of patients who had received anodal tDCS. Finally, there were no significant differences in UR onset latency, UR peak latency, and the number of alpha blinks between the first and second session in both treatment groups.

At the end of the two-week intervention period, 35% of participants correctly identified the stimulation condition to which they had been allocated. Distributions of thoughts about treatment assignment did not differ between both groups ($p = 0.44$), as assessed by a chi-square test, indicating successful blinding.

4. Discussion

This study examined delay eyeblink conditioning in SCA3 patients before and after a two-week regimen of daily cerebellar anodal tDCS or sham tDCS sessions and has three main findings. First, a comparison with healthy controls at baseline revealed significantly fewer conditioned eyeblink responses and longer onset and peak latencies of CRs in SCA3 patients. Second, there were significant correlations between both timing parameters and disease duration, indicating a shift of CRs toward US onset as disease duration increases in SCA3. By contrast, CR acquisition was inversely associated with the number of failed CCAS-S test items but not with SARA score or disease duration. Third, although cerebellar anodal tDCS did not significantly alter CR acquisition compared to sham stimulation, a treatment effect on CR timing parameters was found. Specifically, anodal tDCS induced a shift of CRs toward US onset, while an opposite pattern was observed in patients who had received sham stimulation.

4.1. Eyeblink conditioning in SCA3 and other cerebellar diseases

The impaired acquisition of CRs in SCA3 patients concurs with previous eyeblink conditioning studies involving individuals with cerebellar disorders and confirms the crucial role of the cerebellum in this motor learning paradigm [27,30–32]. Compared with focal

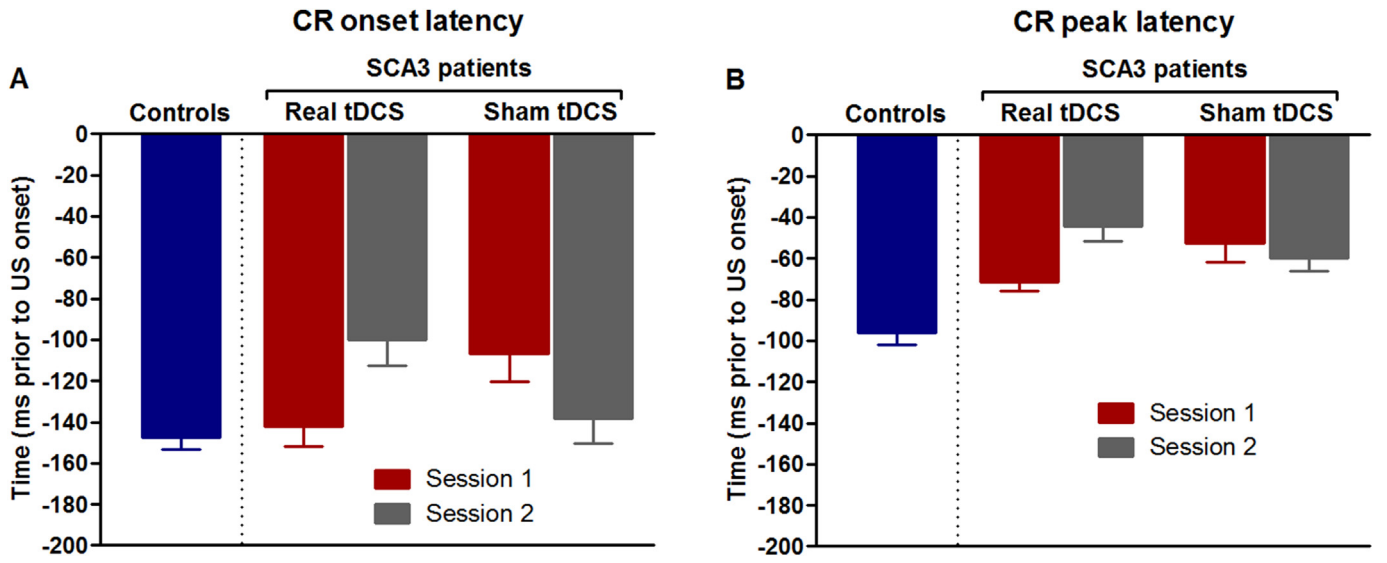

Fig. 4. Timing of conditioned eyeblink responses (CRs) at baseline (red bars) and after ten sessions of cerebellar anodal tDCS or sham tDCS (grey bars) in mildly to moderately affected patients with spinocerebellar ataxia type 3 (SCA3). Shown are means and standard errors of onset (A) and peak latencies (B) of conditioned responses in paired trials. Note that negative values indicate time before presentation of the unconditioned stimulus (US). At two-week follow-up, there were significant between-group differences in onset latency ($p = 0.001$) and peak latency ($p = 0.021$) after adjustment for baseline values. Anodal tDCS induced a shift of CRs toward US onset, while an opposite pattern was observed in patients who had received sham stimulation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

lesions or SCA6, SCA3 is characterized by more diffuse cerebellar pathology, which generally affects output neurons in the deep nuclei to a larger extent than the more superficially located cortex [8]. Relative contributions of cerebellar cortical versus nuclear degeneration to impaired CR acquisition are difficult to ascertain in our patients, stressing an intrinsic limitation of eyeblink conditioning research in individuals with degenerative ataxias [33,34]. Animal studies in the early 1980s conclusively demonstrated that selective lesions in the ipsilateral anterior interposed nucleus permanently abolished CRs [35–38]. Furthermore, although lobule HVI consistently emerged as the most important cortical region for acquisition, effects of lesioning this area have proven to be somewhat more variable, including either a severe impairment or complete CR abolition [39,40]. Finally, the precise timing of CRs has been related to areas in the anterior lobe [41].

To our knowledge, there has been only one study that previously evaluated eyeblink conditioning in a smaller group of SCA3 patients [27]. Similar to our results but in contrast to their data in individuals with SCA6 and Friedreich ataxia, these investigators found a significant increase in CR percentage across blocks, albeit considerably different from healthy controls. Intriguingly, reduced acquisition of CRs has recently been reported in preclinical SCA3 mutation carriers, suggesting early dysfunction of the olivocerebellar circuitry [24].

Converging lines of evidence indicate a principal role for the cerebellum in sensorimotor and perceptual timing [33,41–45]. In ataxia patients, for instance, the characteristic motor symptoms of intention tremor, hypermetria, and dysdiadochokinesis have been related to disturbances in temporal processing [46–49]. Here, we demonstrate differences in CR onset and peak latencies between SCA3 patients and healthy controls, which adds to this literature on impaired timing [33]. Previous eyeblink conditioning data from cerebellar patients with different etiologies are somewhat conflicting, however, with some of them describing shorter CR latencies [3,29] and others reporting longer CR latencies than healthy volunteers [32,50,51]. As opposed to individuals with SCA6 and Friedreich ataxia, the aforementioned study in SCA3 patients did not find significant differences in CR timing parameters compared with healthy controls [27].

4.2. Associations between disease severity measures and eyeblink conditioning performance in SCA3 patients

Motor and cognitive functions within the cerebellum are known to be subserved by distinct subregions with different efferent projections. As a result, dysfunction in both domains reflects involvement of different cerebellar areas and cerebello-cerebral circuits [52,53]. Our eyeblink conditioning data are in good agreement with this generally acknowledged cerebellar topography. Specifically, the inverse association between CR acquisition and the number of CCAS-S failures, but not ataxia severity, could indicate a common source of pathology, e.g. degeneration of lobule HVI and Crus I [3,34]. Indeed, both areas are not only crucial for eyeblink conditioning but are also importantly implicated in various cognitive functions. Timing of CRs, on the other hand, was correlated with disease duration (i.e., the number of years since onset of gait ataxia) but not with cognitive dysfunction, which may imply involvement of the anterior lobe [3,34,41]. In this regard, it is also interesting to note the parallel between longer CR latencies increasing with disease duration in our SCA3 patients and the pathophysiology of hypermetria and intention tremor, which includes a delayed onset of antagonist [47,49] and second agonist EMG activity [46,48], respectively.

We are aware of one previous eyeblink conditioning study that assessed clinico-neurophysiological associations. Similar to our data, these investigators did not find significant correlations between CR acquisition and International Cooperative Ataxia Rating Scale score in patients with degenerative ataxias [27]. Our study, however, is the first to relate disease severity measures to CR timing, thereby also including cognitive performance.

4.3. Effects of cerebellar anodal tDCS on eyeblink conditioning performance in SCA3 patients

The selective modulation of CR timing but not acquisition by cerebellar anodal tDCS in SCA3 patients further supports the notion that both processes rely on different subregions within the cerebellum [3,34]. A similar discrepancy – longer onset and peak latencies compared to the sham condition but an unchanged

acquisition rate – was recently described in healthy adults after a single session of cerebellar tDCS [4,5]. Of note, the temporal features of CRs in our healthy controls were roughly equal to the values reported in these studies [4,5], highlighting the presence of a very narrow window for adaptively-timed conditioned eyeblink responses to occur in individuals with a properly functioning cerebellum. Interestingly, although SCA3 patients in the intervention group had onset latencies at baseline that were comparable to those of healthy controls, repeated sessions of cerebellar anodal tDCS induced a shift of CRs away from this "optimal time frame". Considering the cross-sectionally observed association between disease duration and CR latencies, these modulating effects on timing may be thought of as detrimental (i.e., cerebellar tDCS causes SCA3 patients to behave as individuals with a longer disease duration). Aside from the later appearance of CRs in the intervention group, we found a shift toward CS onset in sham-treated individuals. This finding arguably reflects the effect of a second eyeblink conditioning session without any interference of cerebellar tDCS and suggests that SCA3 patients, upon repeated exposure to this motor learning paradigm, behave like individuals with a shorter disease duration.

The currently observed behavioural change in temporal processing, whether detrimental or beneficial, provides the first empirical evidence that cerebellar tDCS is capable of modifying cerebellar function in SCA3 patients. The exact underlying mechanism at the cellular and circuit level, however, needs further exploration. Accumulating evidence suggests that granule cells, in general, act as pivotal regulators of precise timing operations, transmitting temporal information within the millisecond range to Purkinje cells [33,54]. Although one may therefore hypothesize that cerebellar tDCS modulates CR onset and peak latencies in SCA3 patients by affecting spike initiation of granule cells, it appears more likely, for several reasons, that a change in excitability of Purkinje cells underlies these behavioural observations. First, from an anatomical perspective, the considerably larger Purkinje neurons with their elaborate dendritic trees are located more superficially in the cerebellar cortex than granule cells, rendering them more amenable to the effects of non-invasive brain stimulation. Second, properly timed suppression of Purkinje cell firing in the eyeblink-controlling C3 zone has repeatedly been shown to drive the CR by disinhibition of anterior interposed nuclei [55–57]. Intriguingly, adaptive timing of CRs still occurred when direct stimulation of parallel fibers was used as the conditioned stimulus, implying that Purkinje cells possess an intrinsic timing mechanism independent of time-varying patterns of granule cell activity [43,58]. Third, a computational modelling study that examined the response of various cerebellar neurons to tDCS-induced electric fields showed the largest impact of cerebellar tDCS on Purkinje cells, with only limited effects on the firing activity of granule cells [59]. Taken together, modulation of Purkinje cell spiking may offer a possible explanation for the shift of CR onset and peak latencies following cerebellar tDCS in healthy controls and SCA3 patients. Furthermore, we hypothesize that a lack of modulation of deep nuclear excitability, either due to neuronal degeneration at these sites or insufficient penetration of currents, explains why cerebellar tDCS, on average, did not affect CR acquisition and overall ataxia severity in SCA3 patients [25].

5. Conclusion

The present findings extend previous observations that acquisition and timing of conditioned eyeblink responses, although tightly coupled, appear to be subserved by distinct subregions within the cerebellum. Moreover, our data provide the first evidence that multiple sessions of cerebellar anodal tDCS can influence cerebellar function in mildly to moderately affected SCA3 patients. Future eyeblink conditioning studies should evaluate whether the observed change in temporal processing following cerebellar tDCS is specific for SCA3 or uniform across degenerative ataxias.

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CRediT authorship contribution statement

Roderick P.P.W.M. Maas: Conceptualization, Methodology, Data collection, Formal analysis, Writing – original draft. **Dennis J.L.G. Schutter:** Conceptualization, Writing – review & editing. **Ivan Toni:** Conceptualization, Writing – review & editing. **Dagmar Timmann:** Writing – review & editing. **Bart P.C. van de Warrenburg:** Conceptualization, Funding acquisition, SARA rating, Writing – review and editing.

Declaration of competing interest

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