RESEARCH ARTICLE

Alcohol Improves Cerebellar Learning Deficit in Myoclonus–Dystonia: A Clinical and Electrophysiological Investigation

Anne Weissbach, MD,[1,2] Elisa Werner, MS,[1] Julien F. Bally, MD,[3] Sinem Tunc, MD,[1,2] Sebastian Löns, MD,[1] Dagmar Timmann, MD,[4] Kirsten E. Zeuner, MD,[5] Vera Tadic, MD,[1,2] Norbert Brüggemann, MD,[1,2] Anthony Lang, MD,[3] Christine Klein, MD,[1] Alexander Münchau, MD,[1] and Tobias Bäumer, MD[1]

Objective: To characterize neurophysiological subcortical abnormalities in myoclonus–dystonia and their modulation by alcohol administration.
Methods: Cerebellar associative learning and basal ganglia–brainstem interaction were investigated in 17 myoclonus–dystonia patients with epsilon-sarcoglycan (*SGCE*) gene mutation and 21 age- and sex-matched healthy controls by means of classical eyeblink conditioning and blink reflex recovery cycle before and after alcohol intake resulting in a breath alcohol concentration of 0.08% (0.8g/l). The alcohol responsiveness of clinical symptoms was evaluated by 3 blinded raters with a standardized video protocol and clinical rating scales including the Unified Myoclonus Rating Scale and the Burke–Fahn–Marsden Dystonia Rating Scale.
Results: Patients showed a significantly reduced number of conditioned eyeblink responses before alcohol administration compared to controls. Whereas the conditioning response rate decreased under alcohol intake in controls, it increased in patients (analysis of variance: alcohol state × group, $p = 0.004$). Blink reflex recovery cycle before and after alcohol intake did not differ between groups. Myoclonus improved significantly after alcohol intake ($p = 0.016$). The severity of action myoclonus at baseline correlated negatively with the conditioning response in classical eyeblink conditioning in patients.
Interpretation: The combination of findings of reduced baseline acquisition of conditioned eyeblink responses and normal blink reflex recovery cycle in patients who improved significantly with alcohol intake suggests a crucial role of cerebellar networks in the generation of symptoms in these patients.

ANN NEUROL 2017;00:000–000

Myoclonus–dystonia (M-D) is a movement disorder often caused by mutations in the epsilon-sarcoglycan (*SGCE*) gene.[1] *SGCE* is part of the dystrophin–glycoprotein complex[2] that is highly expressed in the brain, particularly in γ-aminobutyric acidergic (GABAergic) synapses of the cerebellum.[3] Most patients present with a combination of upper body myoclonus and dystonia, predominantly cervical dystonia and writer's cramp.[4] M-D patients report a remarkable improvement of motor symptoms with alcohol.[5]

Neurophysiological studies suggest a subcortical generator underlying motor symptoms in M-D.[6] Additionally, structural,[7] functional,[8] and metabolic studies[9] revealed cerebellar abnormalities in these patients.

There are several blink reflex paradigms that are capable of testing the interaction of different subcortical

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.25035

Received May 31, 2017, and in revised form Jul 29 and Aug 31, 2017. Accepted for publication Aug 31, 2017.

Address correspondence to Dr Bäumer, Institute of Neurogenetics, University of Lübeck, Center for Brain, Behavior, and Metabolism (CBBM), Marie-Curie-Strasse, 23562 Lübeck, Germany. E-mail: tobias.baeumer@neuro.uni-luebeck.de

From the [1]Institute of Neurogenetics, University of Lübeck, Lübeck, Germany; [2]Department of Neurology, University of Lübeck, Lübeck, Germany; [3]Morton and Gloria Shulman Movement Disorder Clinic and Edmond J. Safra Program in Parkinson's Disease, Toronto Western Hospital, University Health Network, University of Toronto, Toronto, Ontario, Canada; [4]Department of Neurology, University of Duisburg–Essen, Duisburg and Essen, Germany; and [5]Department of Neurology, University of Kiel, Kiel, Germany.

pathways. The blink reflex R2-recovery cycle is a noninvasive technique demonstrating abnormal excitability in pontine/medulla oblongata–basal ganglia circuits in patients with dystonia, including M-D, compared to controls.[6,10]

Classical eyeblink conditioning is an associative learning paradigm involving the cerebellar interposed nucleus and cerebellar cortex.[11] Intracortical cerebellar recordings in partly ablated animals,[12] as well as human lesion studies, have shown that lesions within these cerebellar regions result in significant alterations of the acquisition of the conditioning response.[12,13] Additionally, previous animal studies have provided evidence that different forms of neuroplasticity can be induced in these areas.[13]

In M-D, the exact subcortical generator of motor symptoms and their modulation by alcohol is still under debate. To date, there has been no study addressing both clinical and electrophysiological effects of alcohol in *SGCE* mutation-positive M-D. Elucidating these effects not only will be important for an improved understanding of the pathophysiology of M-D and related movement disorders but may also inform the search for new therapeutic targets with a similar effect as alcohol but sparing its addictive potential and side effects.

Subjects and Methods

Participants and Study Design

Seventeen *SGCE* mutation-positive M-D patients (9 female, mean age = 38 ± 16 years, range = 14–59 years) were included. Six of the mutations found in patients were reported previously.[14–16] None of the patients received any centrally effective medication. We also included an age- and sex-matched control group of 21 subjects (11 female, mean age = 36 ± 15 years). Participants were investigated on 2 consecutive days. On day 1, baseline values without alcohol intake (Off C2) were determined, including a standardized video protocol, clinical scales, and measurements of the blink reflex R2-recovery cycle and the classical eyeblink conditioning. On day 2, all measurements were repeated after an oral alcohol administration (On C2) to create a breath alcohol concentration of about 0.08% (0.8g/l). In a control experiment, another 9 healthy subjects (5 female, mean age = 27 ± 5 years) underwent classical eyeblink conditioning on 2 consecutive days without intake of alcohol.

The local ethics committee approved the study, and all subjects gave written informed consent to participate.

Clinical Examinations

Patients underwent a detailed neurological examination including a standardized video protocol of rating scales for myoclonus (Unified Myoclonus Rating Scale [UMRS]) and dystonia (Burke–Fahn–Marsden Rating Scale [BFMDRS], Toronto Western Spasmodic Torticollis Rating Scale [TWSTRS], and Writer's Cramp Rating Scale [WCRS]). Three raters who were blinded to the status of the patients independently evaluated the videos of all M-D patients. Videos were scored in a randomized order, generated by a computer-based randomization tool (www.random.org). Due to the video-based rating system, clinical scores were modified, and sections 2, 4, and 5 of the UMRS, section 1 of the TWSTRS, and the movement scale of the BFMDRS as well as the entire WCRS were used.

For the UMRS, in addition to sections 2, 4, and 5, a summation score of these three sections was calculated (total modified UMRS = mUMRS). In section 4, myoclonus on action is scored during any arm and foot movements. Flexion of the trunk was not videotaped and scored, so that the maximum score of this section was 144 points. The total score of the mUMRS was 300 points (section 2: 128; section 4: 144; section 5: 28).

For the TWSTRS, the effect of a sensory trick was not videotaped and patients were not asked to maintain a neutral position for 60 seconds. Therefore, these parts were not scored and the maximum total modified TWSTRS score (mTWSTRS) was 29 points.

All videos were rated without audio to keep the raters blinded to possible effects of alcohol consumption. Therefore, the speech and swallowing section of the BFMDRS was not rated and the maximum score was 104 points (modified BFMDRS = mBFMDRS).

Blink Reflex R2-Recovery Cycle

The blink reflex R2-recovery cycle was measured using electrical and air puff stimulation according to a previously published protocol.[10] Within the recovery cycle, 2 stimuli per trial were given with interstimulus intervals (ISIs) of 200, 300, 500, 1,000, and 3,000 milliseconds in a randomized order. Each ISI was measured 6 times. The intertrial interval was jittered between 20 and 35 seconds to ensure complete recovery of the blink reflex. Only the right eye was investigated.

To elicit the blink reflex R2-recovery cycle electrically, electrical stimuli were applied to the right supraorbital nerve with Ag/AgCl disk surface electrodes placed over the supraorbital foramen under the eyebrow for optimal nerve stimulation. A constant current stimulator (DS7A Digitimer; Digitimer Limited, Welwyn Garden City, U.K.) was used. To produce a robust reflex response, the intensity of the electrical stimulus was adjusted to 3 times the R2 threshold and applied for 0.2 milliseconds (400V). R2 threshold was defined as the intensity of stimulation needed to produce R2 responses in at least 5 of 10 successive trials in the relaxed orbicularis oculi muscle.

To mechanically elicit a blink reflex, a custom-made air nozzle applied air puffs to the outer canthus of the right eye. The device produced an air puff of 110kPa over 100 milliseconds to the cornea, sufficient to create a robust reflex response.

Classical Eyeblink Conditioning

The classical eyeblink conditioning protocol was adapted from a previously published protocol.[16] Two types of stimuli were used. The air puff (unconditioned stimulus) was preceded by 440 milliseconds by a 1kHz tone (conditioned stimulus) of

88dB sound pressure level with a duration of 540 milliseconds. These pairs of stimuli were applied 100 times in 10 acquisition blocks (AB1–AB10) of 10 paired stimuli each. The intertrial interval was jittered between 20 and 35 seconds to avoid habituation. The 10 acquisition blocks were followed by 3 extinction blocks (EB1–EB3) of 10 stimuli each, where only the conditioned stimulus was applied. Before measuring the classical eyeblink conditioning, participants were familiarized with the conditioned stimulus and the unconditioned stimulus by applying 10 stimuli each in a pseudorandomized order. The conditioned stimulus was presented via earphones that constantly played a white noise signal to mask ambient noises at a 62dB sound pressure level. To maintain the participants' vigilance and attention during the whole experiment, they watched a silent movie.

Electromyographic Recording of Eye Blinks

Electromyographic (EMG) traces of the orbicularis oculi muscle were recorded with 2 Ag/Ag-Cl disk surface electrodes placed over the right orbicularis oculi muscle. The ground electrode was placed at the wrist. EMG signals were amplified and filtered (20Hz and 2kHz) with a D360 amplifier (Digitimer Limited). Signals were sampled at 5kHz, digitized using a laboratory interface (Micro 1401; Cambridge Electronics Design, Cambridge, U.K.), and recorded and stored on a personal computer using SIGNAL 6 software (Cambridge Electronic Devices, Cambridge, U.K.).

Data Analysis

Single trial data were preprocessed. They were direct current subtracted, rectified, and smoothed by a low-pass filter (cuttoff frequency = 0.06Hz). Blink onset was defined as an increase of EMG activation of >130% of the baseline activity for >50 milliseconds.

Data from the blink reflex R2-recovery cycle were processed as described before.[10] The area under the curve of the blink responses was calculated in a time window of 30 to 120 milliseconds after stimulus offset. For each ISI, the relative mean area under the curve of the blink after the second stimulus was calculated in relation to that of the first stimulus. Each trial was verified manually for correct marker settings of the data analysis scripts used.

According to previously published studies,[17] a script for analyzing the classical eyeblink conditioning trials was used. It classified blinks as reflexive alpha blinks if blinks began within 150 milliseconds after the conditioned stimulus, as conditioned response if blinks appeared 150 milliseconds after the conditioned stimulus but before the unconditioned stimulus, and as unconditioned blinks if blinks began up to 250 milliseconds after the unconditioned stimulus. Blinks occurring outside the delineated intervals of each trial were classified as spontaneous blinks. In the extinction phase, conditioned responses were defined as blinks occurring from 150 milliseconds after conditioned stimulus onset up to 150 milliseconds after conditioned stimulus offset.

The conditioned response rate was expressed as a percentage of the conditioned responses within each block of 10 trials of the acquisition phase (AB1–AB10) and the 3 blocks of the extinction phase (EB1–EB3). In addition, the number of spontaneous blinks during the 13 blocks was calculated.

Alcohol Administration

To achieve a breath alcohol concentration of 0.08% (±0.01%), the required amount of ethanol was calculated individually using the Widmark formula[18] in each participant. This formula takes into account the individual factors sex, body weight, and height. Ethanol (96%) was mixed with sugar-free and decaffeinated soda. Participants were asked to drink their portion within at least 30 minutes. Breath alcohol concentration was measured 10 minutes after finishing drinking with the Dräger Alcotest 7510 (Drägerwerk AG & Co. KGaA, Lübeck, Germany). This device is listed on the U.S. National Highway Traffic Safety Administration's Conforming Product List as an evidential breath tester.

All participants completed the Alcohol Use Disorders Identification Test to exclude subjects with alcohol use disorders.

Two patients and 2 healthy controls were <18 years old, and 1 patient suffered from alcohol abuse. These individuals were therefore excluded from alcohol intake and from clinical and electrophysiological measurements under alcohol influence.

Statistical Analysis

Clinical scores were compared before and after alcohol administration using Wilcoxon signed-rank test for paired data. Bonferroni correction was used to correct for multiple comparisons. To measure the agreement between the 3 raters, inter-rater reliability was assessed by the intraclass correlation coefficient (ICC).

The blink reflex measurements were analyzed using a multifactorial repeated measures analysis of variance (ANOVA) to test for differential effects of the factors STATE (Off C2 and On C2), ISI (for blink reflex R2-recovery cycle only), STIMULUS (electrical stimulation and air puff stimulation, for blink reflex R2-recovery cycle only), and BLOCK (acquisition block AB1–AB10; for classical eyeblink conditioning only), as well as the between-subjects factor GROUP (M-D patients and controls). The extinction phase between groups was analyzed for both days including the 3 extinction blocks (EB1–EB3) and the last block of the acquisition phase (AB10) using a repeated measures ANOVA with the factors BLOCK, STATE, and GROUP. Greenhouse–Geisser correction was used to correct for nonsphericity.

To investigate the effects of a repetition of classical eyeblink conditioning and its modulation by alcohol, we compared the 2 groups of healthy subjects using a multifactorial ANOVA with the factors DAY (day 1 and day 2) and BLOCK and the between-subject factor GROUP (controls with alcohol consumption on day 2 and controls without alcohol consumption on day 2).

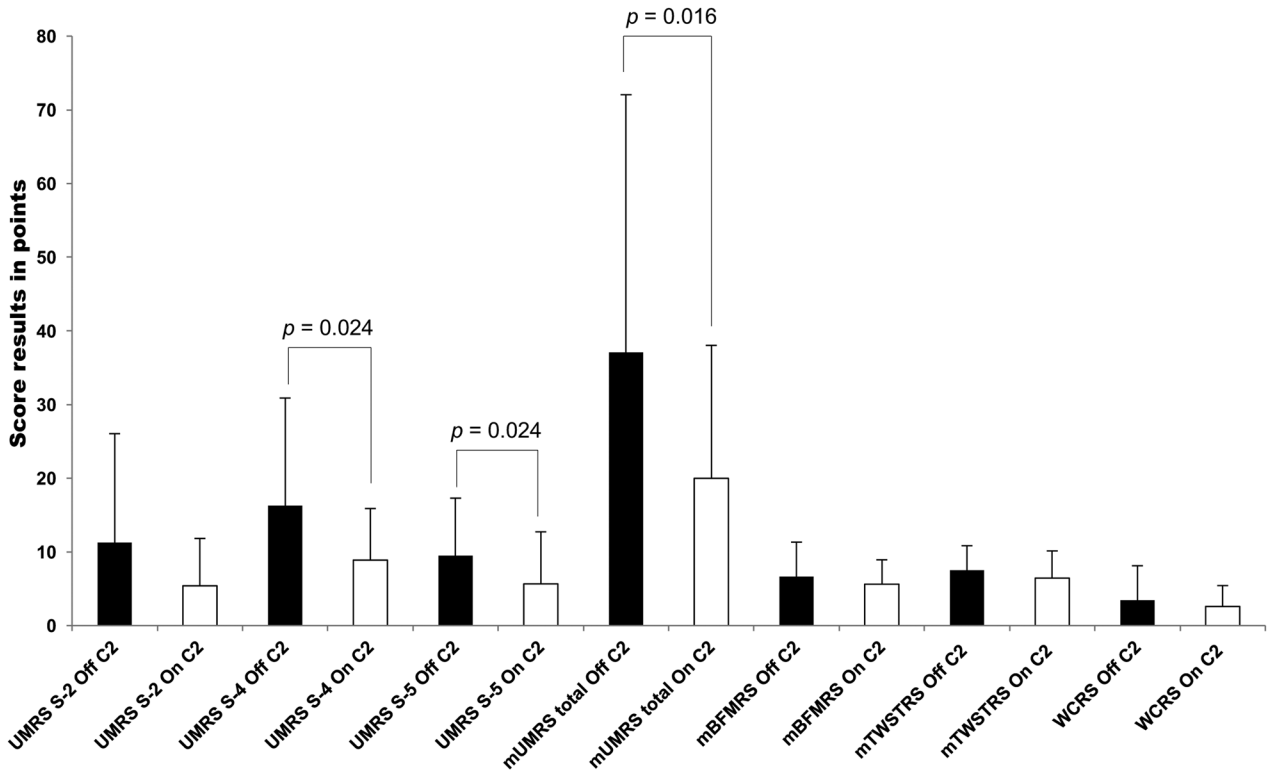

FIGURE 1: Results of the modified Unified Myoclonus Rating Scale (mUMRS; S-2 = section 2, myoclonus at rest; S-4 = section 4, myoclonus during action; S-5 = section 5; functional performance), the modified Burke–Fahn–Marsden Rating Scale (mBFMDRS), the modified Toronto Western Spasmodic Torticollis Rating Scale (mTWSTRS), and the entire Writer's Cramp Rating Scale (WCRS) in myoclonus–dystonia patients before (Off C2) and after (On C2) acute alcohol administration. Bars represent the mean value and whiskers the standard deviation (SD). There was significant improvement of myoclonic jerks after alcohol intake (0.08% ± 0.01% breath alcohol concentration) on section 4 (myoclonus during action: Off C2 mean [SD] = 16 [15], On C2 mean [SD] = 9 [7], $p = 0.024$), on section 5 (functional performance: Off C2 mean [SD] = 10 [8], On C2 mean [SD] = 6 [7], $p = 0.024$), and on the total mUMRS (summation of sections 2, 4, and 5: Off C2 mean [SD] = 37 [35], On C2 mean [SD] = 20 [18], $p = 0.016$; all Bonferroni corrected).

For explorative purposes, we tested for a correlation of clinical characteristics (sex, disease duration, age at examination, and age at onset), clinical improvement (differences of clinical scores before and after alcohol administration), and change of the conditioning response incidence after alcohol administration. A nonparametric correlation analysis (Spearman) was used.

Results

Clinical Findings

The majority of patients presented with myoclonus most pronounced in the neck, trunk, and upper limbs, which was often combined with cervical dystonia and/or writer's cramp, as well as a focal dystonia of the upper limbs. The video ratings revealed an alcohol-associated reduction of myoclonus with action (UMRS section 4, Off C2 mean [standard deviation (SD)] = 16 [15], On C2 mean [SD] = 9 [7]; $p = 0.024$), functional performance (UMRS section 5, Off C2 mean [SD] = 10 [8], On C2 mean [SD] = 6 [7]; $p = 0.024$), and total mUMRS (Off C2 mean [SD] = 37 [35], On C2 mean [SD] = 20 [18]; $p = 0.016$; all Bonferroni corrected) after alcohol administration (Fig 1 and Table). Myoclonus on action was more pronounced than myoclonus at rest (section 2 UMRS). Although myoclonus at rest decreased under alcohol, this reached a statistical trend level only (UMRS section 2, Off C2 mean [SD] = 11 [15], On C2 mean [SD] = 5 [6]; $p = 0.074$). There were no significant changes of the mBFMDRS, the mTWSTRS, and the WCRS scores after alcohol intake.

F1 T1

The inter-rater reliability of the video rating was good, with the highest ICC for the UMRS being ICC = 0.943 (95% confidence interval [CI] = 0.84–0.98) and the lowest ICC for the mTWSTRS being ICC = 0.707 (95% CI = 0.310–0.877).

Electrophysiological Findings

BLINK REFLEX R2-RECOVERY CYCLE. ANOVA revealed a significant effect for the factors ISI ($F_{2.5;66.5} = 67.2$, $p < 0.001$) and STIMULUS ($F_{1;26} = 52.1$, $p < 0.001$), indicating significantly stronger inhibition in patients and controls when being stimulated with electrical stimulation in comparison to air puff stimulation (Fig 2).

F2

J_ID: ANA Customer A_ID: ANA25035 Cadmus Art: ANA25035 Ed. Ref. No.: 17-0635.R2 Date: 15-September-17 Stage: Page: 5

TABLE. Clinical and Genetic Information of Patients

Patient code	Mutation	AAO	AAE	DD	BAC	mUMRS total Off C2	mUMRS total On C2	mBFMRS Off C2	mBFMRS On C2
M-D 1	delEx11	1	14	13	n. a.	11	n. a.	1	n. a.
M-D 2	delEx6	3	49	46	0,7	114	63	11	6
M-D 3	c.304 C>T; p.R102X	1	21	20	0,8	47	11	5	1
M-D 4	delEx6	3	47	44	0,9	61	42	17	11
M-D 5	c.625insG; G209GfsX7	2	18	16	0,8	11	5	2	2
M-D 6	c.625insG; G209GfsX7	47	57	10	0,7	107	43	8	6
M-D 7	c.619delA; p.Arg207GLyfsX11	3	18	15	0,7	28	16	3	2
M-D 8	c.619delA; p.Arg207GLyfsX11	n. a.	50	n. a.	1,0	6	5	2	4
M-D 9	c.304C>T; p.R102X	6	33	27	0,7	34	31	7	7
M-D 10	delEx2	4	14	10	n. a.	32	n. a.	10	n. a.
M-D 11	c.619delA; p.R207fs*15	2	40	38	0,7	11	4	2	6
M-D 12	c.619delA; p.R207fs*15	n. a.	59	n. a.	0,7	9	15	6	5
M-D 13	c.619delA; p.R207fs*15	n. a.	35	n. a.	0,7	11	10	5	2
M-D 14	del(7)(q21.3); arr7q21.3	2	33	31	0,9	16	7	7	6
M-D 15	c.232 + 1G>T; IVS2 + 1G>T	4	51	47	0,7	39	23	15	9
M-D 16	delEx2	8	53	45	n. a.	18	n. a.	5	n. a.
M-D 17	c.549_552del; p.Phe183Leufs*4 (Exon5)	17	49	32	0,9	26	5	5	12

AAO = age of onset in years; AAE = age at examination in years; DD = disease duration in years; BAC = breath alcohol concentration in g/l; UMRS = Unified Myoclonus Rating Scale; BFMDRS = Burke Fahn Marsden Dystonia Rating Scale; Off C2 = without alcohol intake; On C2 = after alcohol intake; n. a. = not available

When the recovery cycle was analyzed separately for each stimulus type, patients and controls showed an effect of ISI (ISI: $F_{2.7;72.5} = 111.8$, $p < 0.001$) in the blink reflex R2-recovery cycle induced by electrical stimulation (see Fig 2). There was no group difference (GROUP: $F_{1;28} = 0.11$, $p = 0.74$). There was also no significant difference after alcohol administration (STATE: $F_{1;27} = 1.19$, $p = 0.29$).

In keeping with the electrical stimulation, there was also a significant effect of ISI (ISI: $F_{2.6;75.5} = 25.05$, $p < 0.001$) in the blink reflex R2-recovery cycle elicited by air puffs. Again, findings did not differ between groups (GROUP: $F_{1;29} = 0.063$, $p = 0.804$). Also, alcohol administration did not cause any significant change (STATE: $F_{1;29} = 0.04$, $p = 0.53$).

CLASSICAL EYEBLINK CONDITIONING. ANOVA of the acquisition phase (AB1–AB10; Fig 3) showed a significant main effect of the factors GROUP ($F_{1;30} = 4.7$, $p = 0.039$) and BLOCK ($F_{9;207} = 23.72$, $p < 0.001$) and an interaction of the factors STATE × GROUP ($F_{1;30} = 9.77$, $p = 0.004$), indicating a general group difference and differential effects between groups after alcohol intake (see Fig 3).

M-D patients showed a significantly reduced conditioned response rate on the first day without alcohol administration (Off C2) compared to controls (ANOVA; GROUP: $F_{1;35} = 10.89$, $p = 0.002$; BLOCK: $F_{9;315} = 21{,}71$, $p < 0.001$).

Importantly, whereas the conditioned response under alcohol administration decreased in controls, it increased in M-D patients, indicating that their cerebellar-dependent associative motor learning was significantly enhanced by the ingestion of alcohol (see Fig 3). A separate ANOVA for each group showed a main effect of the factors BLOCK ($F_{9;117} = 6.6$, $p < 0.001$) and STATE ($F_{1;13} = 9.76$, $p = 0.008$) when analyzing the percentage of conditioned responses before and after

ID: jwweb3b2server Time: 16:02 I Path: //chenas03/Cenpro/ApplicationFiles/Journals/Wiley/ANA#/Vol00000/170136/Comp/APPFile/JW-ANA#170136

J_ID: ANA Customer A_ID: ANA25035 Cadmus Art: ANA25035 Ed. Ref. No.: 17-0635.R2 Date: 15-September-17 Stage: Page: 6

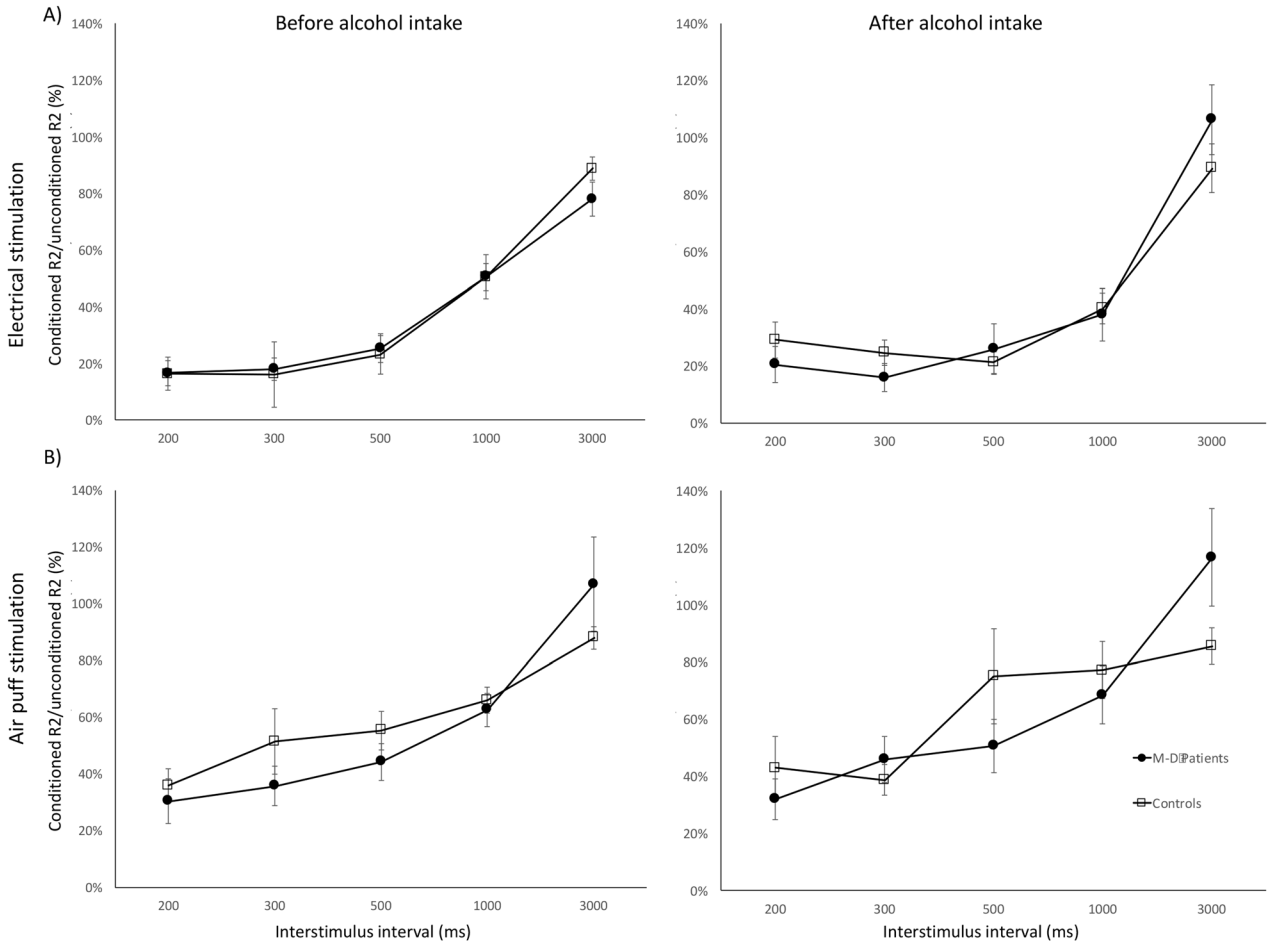

FIGURE 2: Results of the blink reflex R2-recovery cycle obtained with electrical stimulation (A) or induced by air puffs (B). For both techniques, the panels on the left show blink reflex R2-recovery cycle results before alcohol intake, and the panels on the right under alcohol influence. Inhibition during blink reflex R2-recovery cycle was stronger with electrical compared to air puff stimulation. There was no blink reflex R2-recovery cycle group difference and no effect of alcohol administration.

alcohol consumption in M-D patients. In controls, the factor BLOCK ($F_{9;153} = 21.21$, $p < 0.001$) was also significant, but the factor STATE ($F_{1;17} = 2.29$, $p = 0.15$) did not reach significance.

Analyzing the extinction phase (EB1–EB3) and the last acquisition block (AB10), the factors STATE ($F_{1;30} = 0.22$, $p = 0.882$) and GROUP ($F_{1;30} = 3.395$, $p = 0.075$) had no influence. The factor BLOCK ($F_{3;90} = 32.75$, $p < 0.001$) and the interaction of factors STATE × BLOCK × GROUP ($F_{3;90} = 3.19$, $p = 0.034$) were, however, significant. This interaction was mainly driven by the two groups differing to a great extent at the end of the acquisition phase (AB10) on day 1 rather than due to a difference of values in the extinction phase (Fig 4). F4

Comparing the eyeblink conditioning of healthy controls with and without alcohol consumption, ANOVA revealed a significant interaction of the factors DAY × GROUP ($F_{1;25} = 8.175$, $p = 0.008$). A separate analysis of the second day showed a significant effect of the factor GROUP ($F_{1;25} = 6.741$, $p = 0.016$), illustrating that alcohol intake abolished the physiological increase of eyeblink conditioning on day 2 (Fig 5). Healthy controls without alcohol influence showed a significantly stronger conditioned response on day 2 compared to day 1 ($p = 0.016$). There was no significant difference of the extinction phase between groups on day 1 or 2. F5

Clinical Correlations

The explorative correlation showed a negative correlation of myoclonus during action (section 4 of the UMRS) before alcohol intake with the percentage of conditioned responses during acquisition of the classical eyeblink conditioning (uncorrected values: AB6, $r = -0.551$, $p = 0.022$; AB7, $r = -0.508$, $p = 0.037$; AB9, $r = -0.504$, $p = 0.039$), indicating reduced eyeblink conditioning in the acquisition phase in M-D patients with more severe action myoclonus.

Moreover, the total mUMRS and the mBFMDRS before alcohol administration correlated with the change of these clinical scores after alcohol intake (uncorrected

ID: jwweb3b2server Time: 16:02 I Path: //chenas03/Cenpro/ApplicationFiles/Journals/Wiley/ANA#/Vol00000/170136/Comp/APPFile/JW-ANA#170136

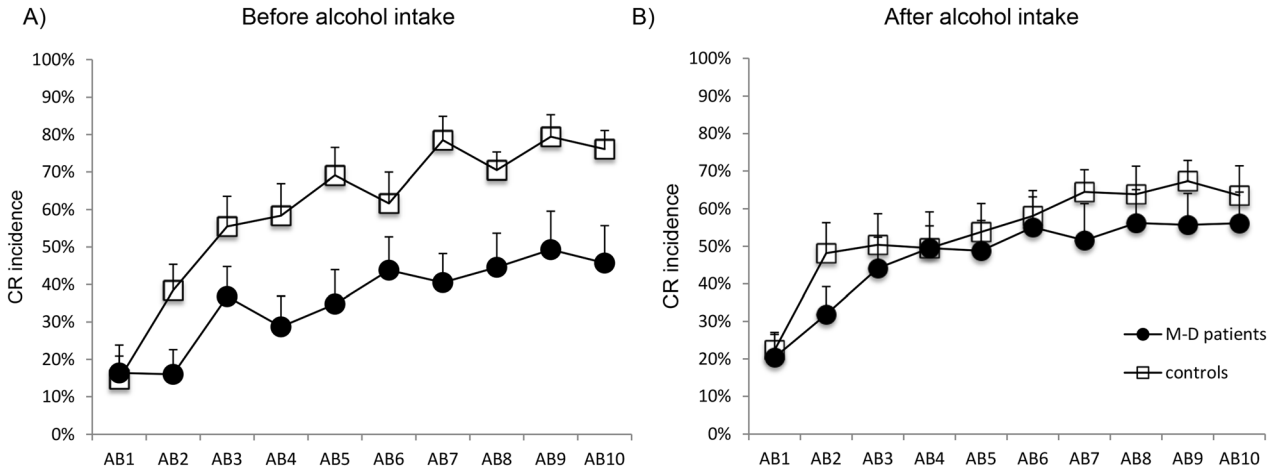

FIGURE 3: Results of the acquisition phase of the classical eyeblink conditioning on the first day before (A) and on the second day after acute alcohol administration (B) in myoclonus–dystonia (M-D) patients and healthy control subjects. The number of conditioning responses (CR) per acquisition block is significantly reduced in M-D patients compared to controls on day 1 ($p = 0.002$). After alcohol intake on day 2, the conditioned response significantly increased compared to day 1 in patients ($p = 0.008$) and was then similar to that in healthy controls (whose responses decreased) on day 2. AB = acquisition block.

values: total UMRS, $r = 0.708$, $p = 0.005$; mBFMDRS, $r = 0.579$, $p = 0.03$), suggesting that M-D patients with more severe myoclonic jerks and dystonia had a stronger alcohol responsiveness.

Discussion

This is the first study to address the effects of alcohol on clinical and neurophysiological parameters of brainstem excitability and cerebellum-dependent associative motor learning in M-D patients and healthy controls.

The main findings of our study are that (1) classical eyeblink conditioning as a measure of cerebellar-dependent associative motor learning is reduced in M-D; and (2) in contrast to controls, it improves rather than deteriorates after alcohol administration.

Eyeblink conditioning is mediated by olivopontocerebellar circuits, predominantly involving pontine nuclei, inferior olive, cerebellar interposed nucleus, and lobule VI of the cerebellar cortex.[12,13] Alterations have previously been reported in another cohort of M-D patients, with the acquisition phase being normal, but the following extinction phase being attenuated.[19] The differences between these abnormalities in eyeblink conditioning compared to our findings might be explained by methodological differences. Specifically, the previous study[19] used electrical stimulation of the supraorbital nerve but not air puff and included fewer acquisition and extinction blocks with a different number of individual trials. Also, their patients were ~10 years younger than ours. All of these factors may have a substantial influence on the conditioning response.[20]

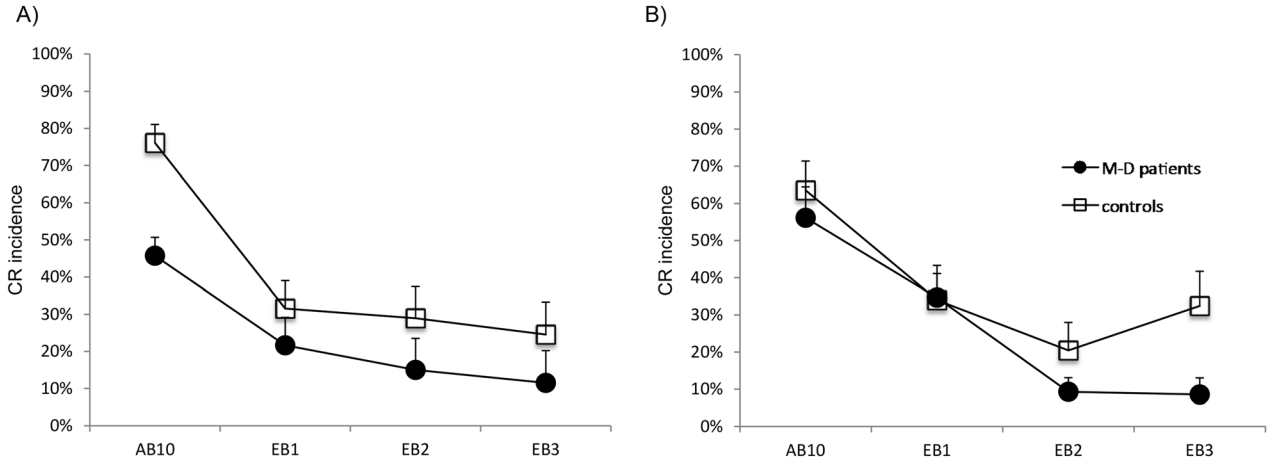

FIGURE 4: Results of acquisition block 10 (AB10) and extinction block 1–3 (EB1–EB3) of the classical eyeblink conditioning on the first day before (A) and on the second day after acute alcohol administration (B) in myoclonus-dystonia (M-D) patients and healthy control subjects. There was a significant effect for the factor BLOCK ($F_{3;90} = 32.75$, $p < 0.001$) and a significant interaction of the factors STATE × BLOCK × GROUP ($F_{3;90} = 3.19$, $p = 0.034$). This interaction resulted mainly from the difference between the groups at AB10 on day 1, but not in the extinction phase itself. CR = conditioning response.

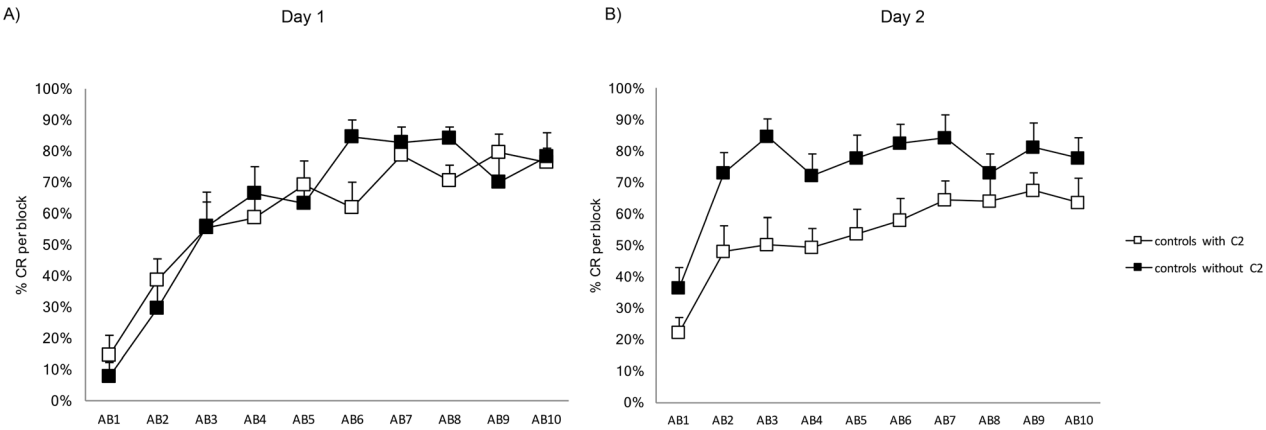

FIGURE 5: Results of the control experiment. The conditioned response (CR) rate per acquisition block (AB) of the classical eyeblink conditioning on 2 consecutive days in a group of healthy controls without alcohol consumption (controls without C2) and under alcohol intake on day 2 (controls with C2) is shown. Controls without alcohol influence had significantly higher CRs on the second day compared to patients who received alcohol ($p = 0.016$).

In contrast to the eyeblink conditioning results, the blink reflex R2-recovery cycle was normal in our M-D patients, suggesting normal activity in pontine/medulla oblongata–basal ganglia circuits. This contrasts with the decrease of inhibition of the recovery cycle obtained in a number of conditions, including 6-hydroxydopamine lesioned rat models of parkinsonism,[21] patients with genetically undefined, untreated Parkinson disease,[22] and patients with blepharospasm[10] or cervical dystonia,[23] all in keeping with the assumption of disinhibition within these circuits.[24] In a previous study investigating the blink reflex R2-recovery cycle with electrical stimulation in M-D, a similar enhancement of the blink reflex R2-recovery cycle was found.[6] Importantly, in that study,[6] healthy control subjects showed a stronger inhibition compared to controls in our and other previously published studies,[10] whereas their M-D patients presented with an inhibition comparable to the control groups in our and other studies.[10] In our M-D patients, the blink reflex R2-recovery cycle was normal when investigated both with electrical stimulation and with air puffs.

Normal blink reflex R2-recovery cycle findings in our cohort, along with altered classical eyeblink conditioning, suggest that the neurophysiological abnormalities preferentially affect cerebellar networks in our cohort of *SGCE* mutation-positive M-D patients. Interestingly, this notion is corroborated by a functional magnetic resonance imaging study of M-D patients revealing cerebellar hyperactivity during a finger movement task.[8] Additionally, metabolic increases of the parasagittal cerebellum were found in a (^{18}F)-fluorodeoxyglucose positron emission tomography study in M-D patients, as well as in posthypoxic myoclonus patients.[9] Moreover, a structural imaging study using voxel-based morphometry and diffusion tensor imaging showed increased white matter volume and fractional anisotropy and decreased mean diffusivity in the subthalamic area of the brainstem connecting the cerebellum with the thalamus.[7] In general, cerebellar alterations in different types of genetic[25] and sporadic dystonia[26] identified with various imaging[27] as well as electrophysiological techniques[28–30] and their pathophysiological contribution have been a matter of debate.[31,32] Traditionally, models of the pathophysiology of dystonia have focused on the dominant role of the basal ganglia and its connections.[33] More recently, increasing evidence has emerged pointing toward the concept of a network disorder with multiple nodes being involved in the pathophysiology of dystonia, including the basal ganglia, sensorimotor cortex, thalamus, and cerebellum.[31] In this network, the cerebellum (and cerebellothalamic fibers) especially appears to play an important role in the pathophysiology of a subgroup of dystonia,[34,35] but does not seem to be the sole culprit but rather a contributing partner within altered sensorimotor and basal ganglia circuits. In contrast to other disorders with cerebellar pathology where ataxia prevails, the cerebellar abnormality in dystonia distorts and increases rather than abolishes cerebellar output.[36] Lesion studies provide further evidence that local irritation rather than destruction with a loss of function, and pathologic long-term adaptive responses from cerebellar areas near the lesion, cause the altered cerebellar function.[36,37] At a neurophysiological level, these cerebellar abnormalities result in disinhibition of dentatothalamocortical pathways and a change of afferent sensory signal integration important for controlled motor output. However, pathologic cerebellar contribution is suggested to be of variable intensity in different dystonic subtypes.[31] Interestingly,

patients with essential tremor, in whom cerebellar deficits have been demonstrated, also show a significant reduction of symptoms under alcohol influence.[38]

Previous studies have reported that alcohol functions as a GABA agonist, but also other, mostly subcortical neurotransmitters such as serotonin, dopamine, glutamate, cannabinoid, and beta-endorphin are targets of even low doses of ethanol.[39] In addition, alcohol significantly reduces brain glucose metabolism, but increases the metabolism of acetate, which is a metabolite of alcohol.[39] Intriguingly, this effect was particularly evident in the cerebellum. It is tempting to speculate that these aspects also contribute to reduced eyeblink conditioning rates, as well as to the reduced myoclonic symptoms in our patients.

Regarding M-D, the *SGCE* gene is highly expressed in the cerebellum,[40] especially in GABAergic synapses of Purkinje cells.[3] They function as a relay of incoming climbing fibers, processing unconditioned stimulus signals, and mossy as well as parallel fibers, carrying afferent conditioned stimulus signals, and then transfer this information toward the interposed nucleus.[12] Mutations in the *SGCE* gene probably cause a GABAergic deficit due to Purkinje cell dysfunction that results in a disinhibition of classical eyeblink conditioning pathways. Animal studies suggested that during classical eyeblink conditioning, plasticity occurs at the cerebellar cortical level as well as within the interposed nucleus. To date, multiple synaptic and intrinsic forms of plasticity have been described, underlying acquisition and retention of conditioned eyeblinks.[41] Apparently, *SGCE* mutations lead to alterations in these cerebellar circuits in M-D patients.

Interestingly and somewhat counterintuitively, after one-time alcohol intake the percentage of conditioned responses increased significantly in our patients; indicating that alcohol improved learning. In contrast, chronic alcohol abuse leads to pronounced disturbances of classical eyeblink conditioning both in adults and children suffering from fetal alcohol syndrome.[42]

Ethanol specifically enhances the inhibitory effect of GABAergic transmission in animals[43] as well as humans.[44] A direct injection of gabazine, a GABA blocker, into the interposed nuclei of the intermediate cerebellum in rabbits abolishes conditioned eyeblink responses.[45] Therefore, one-time alcohol administration enhanced conditioning response acquisition in our M-D patients probably through increasing GABAergic transmission and resetting dysfunctional disinhibition. Presumably, GABAergic deficits associated with *SGCE* mutations were temporarily compensated for by acute alcohol intake. It is tempting to speculate that in healthy control subjects, functioning at the GABAergic optimum, alcohol will cause supraphysiologically increased GABAergic inhibitory influences and thus negatively affect cerebellar-mediated learning. This view is supported by the finding that in healthy rats an infusion of the $GABA_B$ receptor agonist baclofen in the interposed nucleus abolished the conditioning response, although the rats were already trained to the classical eyeblink conditioning paradigm,[46] similar to our healthy subjects on their second day of measurements. Interestingly, in healthy subjects, a repetition of classical eyeblink conditioning results in a significant increase of the eyeblink conditioning.[47] In our control group, alcohol decreased the conditioning rate nonsignificantly. We therefore speculate that alcohol intake dampens the physiological increase of cerebellar-associated learning after repetition of classical eyeblink conditioning, rather than decreasing the conditioned response itself.

GABAergic drugs including benzodiazepines, sodium oxybate, or zonisamide can improve clinical symptoms in patients with myoclonus[48] or M-D,[48–50] but also have high rates of side effects and carry a risk of dependency.[50] Several studies reported improvement of myoclonus at rest before improvement of action myoclonus.[48] In our cohort and as typically seen in M-D, patients had more pronounced myoclonus with action than at rest, likely explaining why myoclonus at rest only improved nonsignificantly in our patients.

When comparing clinical effects of alcohol in our cohort with the impact of other drugs, several differences and limitations need to be considered. First, the patients could not be blinded to their condition, as no placebo arm was included in our study. Second, all patients were investigated only once, having a breath alcohol concentration of about 0.08%. Some patients reported a stronger decrease of myoclonic jerks after previous consumptions of higher amounts of alcohol. This notwithstanding, the present study attempted to systematically and objectively assess the clinical effect of alcohol in *SGCE* mutation-positive M-D and the frequently reported alcohol responsiveness with the help of a standardized, blinded video rating. In a recently published investigation of 18 *SGCE* mutation-positive M-D patients, zonisamide was most effective against action myoclonus (section 4) and improved functional test results (section 5),[49] similar to the effects of alcohol reported here. However, when comparing the clinical response of a given medication with the alcohol response in patients, it is important to keep in mind the strong addiction potential and other grave short- and long-term side effects of alcohol.

To summarize, our study shows that the administration of alcohol increases cerebellar-dependent associative motor learning in M-D patients. Electrically and air puff–induced blink reflex R2-recovery cycle was normal and did not change after alcohol intake. These findings provide further evidence for possible cerebellar abnormalities in *SGCE* mutation-positive M-D patients and might function as a model that opens the door to new pathophysiological concepts and treatment strategies in dystonia.

Acknowledgment

This work was supported by the Deutsche Forschungsgemeinschaft (DFG; SFB 936/project C5, C.K., A.M.) and German Bundesministerium für Bildung und Forschung (BMBF) through a project grant (Dystonia Translational Research and Therapy Consortium [DysTract]). A.W. received a scholarship from the DFG (WE5919/1-1) and a habilitation stipend from the University of Lübeck (H03-2016). K.E.Z. has been supported by the DFG, University of Schleswig Holstein, and Benign Essential Blepharospasmus Foundation. N.B. was funded by the Collaborative Center for X-linked Dystonia-Parkinsonism. A.L. received grants from Brain Canada, Canadian Institutes of Health Research, Edmond J. Safra Philanthropic Foundation, Michael J. Fox Foundation, Ontario Brain Institute, National Parkinson Foundation, Parkinson Society Canada, and W. Garfield Weston Foundation. C.K. is the recipient of a career development award from the Hermann and Lilly Schilling Foundation. A.M. receives support from the Possehl-Stiftung, Lübeck; the Margot und Jürgen Wessel Stiftung, Lübeck; the DFG (SFB 936, project C5), and the BMBF (DysTract consortium, the Innovationsausschuss of the Gemeinsamer Bundesausschuss: Translate NAMSE; structural support for the Lübeck Center for Rare Diseases).

We thank C. Westphal and S. Schmaljohann from Dräger for providing the Dräger Alcotest 7510 (Drägerwerk AG & Co. KGaA) to measure the breath alcohol concentration in our study participants.

Author Contributions

A.W., A.M., J.F.B., A.L., C.K., and T.B. contributed to conception and design of the study. All authors contributed to acquisition and analysis of data. A.W., A.M., and T.B. drafted a significant portion of the manuscript or figures.

Potential Conflicts of Interest

Nothing to report.

References

1. Zimprich A, Grabowski M, Asmus F, et al. Mutations in the gene encoding epsilon-sarcoglycan cause myoclonus-dystonia syndrome. Nat Genet 2001;29:66–69.
2. Waite AJ, Carlisle FA, Chan YM, Blake DJ. Myoclonus dystonia and muscular dystrophy: ε-sarcoglycan is part of the dystrophin-associated protein complex in brain. Mov Disord 2016;31:1694–1703.
3. Ritz K, van Schaik BD, Jakobs ME, et al. SGCE isoform characterization and expression in human brain: implications for myoclonus-dystonia pathogenesis? Eur J Hum Genet 2011;19:438–444.
4. Nardocci N. Myoclonus-dystonia syndrome. Handb Clin Neurol 2011;100:563–575.
5. Hess CW, Raymond D, Aguiar Pde C, et al. Myoclonus-dystonia, obsessive-compulsive disorder, and alcohol dependence in SGCE mutation carriers. Neurology 2007;68:522–524.
6. Marelli C, Canafoglia L, Zibordi F, et al. A neurophysiological study of myoclonus in patients with DYT11 myoclonus-dystonia syndrome. Mov Disord 2008;23:2041–2048.
7. van der Meer JN, Beukers RJ, van der Salm SM, et al. White matter abnormalities in gene-positive myoclonus-dystonia. Mov Disord 2012;27:1666–1672.
8. Beukers RJ, Foncke EM, van der Meer JN, et al. Functional magnetic resonance imaging evidence of incomplete maternal imprinting in myoclonus-dystonia. Arch Neurol 2011;68:802–805.
9. Carbon M, Raymond D, Ozelius L, et al. Metabolic changes in DYT11 myoclonus-dystonia. Neurology 2013;80:385–391.
10. Schwingenschuh P, Katschnig P, Edwards MJ, et al. The blink reflex recovery cycle differs between essential and presumed psychogenic blepharospasm. Neurology 2011;76:610–614.
11. Bracha V, Zbarska S, Parker K, et al. The cerebellum and eye-blink conditioning: learning versus network performance hypotheses. Neuroscience 2009;162:787–796.
12. McCormick DA, Clark GA, Lavond DG, Thompson RF. Initial localization of the memory trace for a basic form of learning. Proc Natl Acad Sci U S A 1982;79:2731–2735.
13. Gerwig M, Kolb FP, Timmann D. The involvement of the human cerebellum in eyeblink conditioning. Cerebellum 2007;6:38–57.
14. Grunewald A, Djarmati A, Lohmann-Hedrich K, et al. Myoclonus-dystonia: significance of large SGCE deletions. Hum Mutat 2008; 29:331–332.
15. Muller B, Hedrich K, Kock N, et al. Evidence that paternal expression of the epsilon-sarcoglycan gene accounts for reduced penetrance in myoclonus-dystonia. Am J Hum Genet 2002;71:1303–1311.
16. Weissbach A, Kasten M, Grunewald A, et al. Prominent psychiatric comorbidity in the dominantly inherited movement disorder myoclonus-dystonia. Parkinsonism Relat Disord 2013;19:422–425.
17. Zuchowski ML, Timmann D, Gerwig M. Acquisition of conditioned eyeblink responses is modulated by cerebellar tDCS. Brain Stimul 2014;7:525–531.
18. Widmark EMP. Die theoretischen Grundlagen und die praktische Verwendbarkeit der gerichtlich-medizinischen Alkoholbestimmung. Berlin, Germay: Urban & Schwarzenberg, 1932.
19. Popa T, Milani P, Richard A, et al. The neurophysiological features of myoclonus-dystonia and differentiation from other dystonias. JAMA Neurol 2014;71:612–619.
20. Bellebaum C, Daum I. Effects of age and awareness on eyeblink conditional discrimination learning. Behav Neurosci 2004;118: 1157–1165.
21. Basso MA, Strecker RE, Evinger C. Midbrain 6-hydroxydopamine lesions modulate blink reflex excitability. Exp Brain Res 1993;94: 88–96.

22. Kimura J. Disorder of interneurons in parkinsonism. The orbicularis oculi reflex to paired stimuli. Brain 1973;96:87–96.
23. Antelmi E, Di Stasio F, Rocchi L, et al. Impaired eye blink classical conditioning distinguishes dystonic patients with and without tremor. Parkinsonism Relat Disord 2016;31:23–27.
24. Aramideh M, Eekhof JL, Bour LJ, et al. Electromyography and recovery of the blink reflex in involuntary eyelid closure: a comparative study. J Neurol Neurosurg Psychiatry 1995;58:692–698.
25. Carbon M, Kingsley PB, Tang C, et al. Microstructural white matter changes in primary torsion dystonia. Mov Disord 2008;23:234–239.
26. Delmaire C, Vidailhet M, Elbaz A, et al. Structural abnormalities in the cerebellum and sensorimotor circuit in writer's cramp. Neurology 2007;69:376–380.
27. Eidelberg D, Moeller JR, Ishikawa T, et al. The metabolic topography of idiopathic torsion dystonia. Brain 1995;118(pt 6):1473–1484.
28. Hubsch C, Roze E, Popa T, et al. Defective cerebellar control of cortical plasticity in writer's cramp. Brain 2013;136(pt 7):2050–2062.
29. Teo JT, van de Warrenburg BP, Schneider SA, et al. Neurophysiological evidence for cerebellar dysfunction in primary focal dystonia. J Neurol Neurosurg Psychiatry 2009;80:80–83.
30. Bologna M, Paparella G, Fabbrini A, et al. Effects of cerebellar theta-burst stimulation on arm and neck movement kinematics in patients with focal dystonia. Clin Neurophysiol 2016;127:3472–3479.
31. Shakkottai VG, Batla A, Bhatia K, et al. Current Opinions and Areas of Consensus on the Role of the Cerebellum in Dystonia. Cerebellum 2017;16:577–594.
32. Prudente CN, Hess EJ, Jinnah HA. Dystonia as a network disorder: what is the role of the cerebellum? Neuroscience 2014;260:23–35.
33. Berardelli A, Rothwell JC, Hallett M, et al. The pathophysiology of primary dystonia. Brain 1998;121(pt 7):1195–1212.
34. Filip P, Lungu OV, Bares M. Dystonia and the cerebellum: a new field of interest in movement disorders? Clin Neurophysiol 2013;124:1269–1276.
35. Zoons E, Tijssen MA. Pathologic changes in the brain in cervical dystonia pre- and post-mortem—a commentary with a special focus on the cerebellum. Exp Neurol 2013;247:130–133.
36. Neychev VK, Gross RE, Lehericy S, et al. The functional neuroanatomy of dystonia. Neurobiol Dis 2011;42:185–201.
37. LeDoux MS, Lorden JF. Abnormal cerebellar output in the genetically dystonic rat. Adv Neurol 1998;78:63–78.
38. Deuschl G, Raethjen J, Hellriegel H, Elble R. Treatment of patients with essential tremor. Lancet Neurol 2011;10:148–161.
39. Volkow ND, Wiers CE, Shokri-Kojori E, et al. Neurochemical and metabolic effects of acute and chronic alcohol in the human brain: studies with positron emission tomography. Neuropharmacology 2017;122:175–188.
40. Xiao J, Vemula SR, Xue Y, et al. Role of major and brain-specific Sgce isoforms in the pathogenesis of myoclonus-dystonia syndrome. Neurobiol Dis 2017;98:52–65.
41. Hansel C, Linden DJ, D'Angelo E. Beyond parallel fiber LTD: the diversity of synaptic and non-synaptic plasticity in the cerebellum. Nat Neurosci 2001;4:467–475.
42. Cheng DT, Jacobson SW, Jacobson JL, et al. Eyeblink classical conditioning in alcoholism and fetal alcohol spectrum disorders. Front Psychiatry 2015;6:155.
43. Nestoros JN. Ethanol specifically potentiates GABA-mediated neurotransmission in feline cerebral cortex. Science 1980;209:708–710.
44. Ziemann U, Lonnecker S, Paulus W. Inhibition of human motor cortex by ethanol. A transcranial magnetic stimulation study. Brain 1995;118(pt 6):1437–1446.
45. Parker KL, Zbarska S, Carrel AJ, Bracha V. Blocking GABAA neurotransmission in the interposed nuclei: effects on conditioned and unconditioned eyeblinks. Brain Res 2009;1292:25–37.
46. Ramirez OA, Nordholm AF, Gellerman D, et al. The conditioned eyeblink response: a role for the GABA-B receptor? Pharmacol Biochem Behav 1997;58:127–132.
47. Gerwig M, Guberina H, Esser AC, et al. Evaluation of multiple-session delay eyeblink conditioning comparing patients with focal cerebellar lesions and cerebellar degeneration. Behav Brain Res 2010;212:143–151.
48. Frucht SJ, Bordelon Y, Houghton WH, Reardan D. A pilot tolerability and efficacy trial of sodium oxybate in ethanol-responsive movement disorders. Mov Disord 2005;20:1330–1337.
49. Hainque E, Vidailhet M, Cozic N, et al. A randomized, controlled, double-blind, crossover trial of zonisamide in myoclonus-dystonia. Neurology 2016;86:1729–1735.
50. Raymond D, Ozelius L. Myoclonus-dystonia. GeneReviews 2003. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1414/.