Repetitive ocular vestibular evoked myogenic potential stimulation for the diagnosis of myasthenia gravis: Optimization of stimulation parameters

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Abstract: OBJECTIVE To determine the most effective stimulation parameters for the diagnosis of ocular myasthenia gravis (MG) using repetitive ocular vestibular evoked myogenic potentials (oVEMP) for quantification of the extraocular muscle response decrement. METHODS Repetitive bone-conducted oVEMPs were elicited in 18 MG patients and 20 healthy subjects. We compared four different stimulus repetition rates (20 Hz, 30 Hz, 40 Hz, 50 Hz) and 100 Hz continuous stimulation, as well as recordings from the inferior oblique muscles and the lateral rectus muscles to determine the most sensitive and specific oVEMP parameters for decrement detection. RESULTS Repetitive stimulation at all tested repetition rates with recordings from inferior oblique muscles allowed for effective differentiation between MG patients and healthy subjects. Among all repetition rates, 30 Hz showed a trend towards superiority, with a sensitivity of 71% and a specificity of 94% (area under the curve (AUC) 0.88) when using the smaller decrement of the two eyes and -10% as cutoff. Considering the larger decrement for analysis (-9% as cutoff), sensitivity increased to 82%, but specificity decreased to 78% (AUC 0.81). CONCLUSIONS Our study demonstrates, that repetitive oVEMP stimulation elicits a robust decrement in the inferior oblique muscles of MG patients at repetition rates between 20 Hz and 50 Hz, with a probable optimum at 30 Hz. SIGNIFICANCE Repetitive inferior oblique oVEMP stimulation with optimal stimulus parameters facilitates early and accurate diagnosis of ocular MG.

DOI: https://doi.org/10.1016/j.clinph.2019.03.033

Clinical Neurophysiology 130 (2019) 1125–1134

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

Repetitive ocular vestibular evoked myogenic potential stimulation for the diagnosis of myasthenia gravis: Optimization of stimulation parameters

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ARTICLE INFO

Article history:
Accepted 30 March 2019
Available online 25 April 2019

Keywords:
oVEMP
Ocular myasthenia gravis
Repetitive stimulation
Decrement
Vestibulo-ocular reflex
Otolith

HIGHLIGHTS

- Repetitive oVEMPs reliably differentiate MG patients from controls.
- Inferior oblique muscle recordings at 30Hz were most sensitive and specific.
- At higher repetition rates, the decrement occurred with later stimulus repetitions.

ABSTRACT

Objective: To determine the most effective stimulation parameters for the diagnosis of ocular myasthenia gravis (MG) using repetitive ocular vestibular evoked myogenic potentials (oVEMP) for quantification of the extraocular muscle response decrement.
Methods: Repetitive bone-conducted oVEMPs were elicited in 18 MG patients and 20 healthy subjects. We compared four different stimulus repetition rates (20 Hz, 30 Hz, 40 Hz, 50 Hz) and 100 Hz continuous stimulation, as well as recordings from the inferior oblique muscles and the lateral rectus muscles to determine the most sensitive and specific oVEMP parameters for decrement detection.
Results: Repetitive stimulation at all tested repetition rates with recordings from inferior oblique muscles allowed for effective differentiation between MG patients and healthy subjects. Among all repetition rates, 30 Hz showed a trend towards superiority, with a sensitivity of 71% and a specificity of 94% (area under the curve (AUC) 0.88) when using the smaller decrement of the two eyes and −10% as cutoff. Considering the larger decrement for analysis (−9% as cutoff), sensitivity increased to 82%, but specificity decreased to 78% (AUC 0.81).
Conclusions: Our study demonstrates, that repetitive oVEMP stimulation elicits a robust decrement in the inferior oblique muscles of MG patients at repetition rates between 20 Hz and 50 Hz, with a probable optimum at 30 Hz.
Significance: Repetitive inferior oblique oVEMP stimulation with optimal stimulus parameters facilitates early and accurate diagnosis of ocular MG.

1. Introduction

Myasthenia gravis (MG) is an acquired chronic autoimmune disorder affecting neuromuscular transmission (Silvestri and Wolfe, 2012). Up to 85% of MG patients initially present with isolated ocular symptoms, such as fluctuating ptosis or diplopia (Kaminski et al., 1990, Barton and Fouladvand, 2000). Early diagnosis of MG is of utmost importance, since this disease represents a potentially life-threatening condition and effective therapeutic options are increasingly available. Particularly in patients with fluctuating, isolated ocular involvement, early and accurate diagnosis of MG often is a challenge. For instance, serological testing,

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https://doi.org/10.1016/j.clinph.2019.03.033
1388-2457/

including antibodies against acetylcholine receptor (AChR-Ab), muscle-specific kinase (MuSK-Ab), titin and low-density lipoprotein receptor-related protein 4 (LRP4-Ab), are mostly negative in isolated ocular MG (Benatar, 2006; Oger and Frykman, 2015). Edrophonium testing bears the risk of relevant adverse events, and repetitive nerve stimulation of peripheral muscles has low sensitivity. Single-fiber electromyography (SFEMG), the current gold standard diagnostic technique, is time-consuming, requires a trained examiner and is often unavailable outside specialized centers (Smith and Lee, 2017). The predilection of MG to involve extraocular muscles led to several attempts to diagnose the disease using eye movement measurements (Barton, 1998). Recently, Valko et al. developed repetitive stimulation with ocular vestibular evoked myogenic potentials (oVEMPs) as a non-invasive diagnostic tool for MG, enabling direct recording of the response decrement from the extraocular muscles (Valko et al., 2016). oVEMPs reflect extraocular muscle activity in response to otolith stimulation (Weber et al., 2012) elicited by either bone-conducted stimulation or air-conducted sound. In recent years, oVEMPs have gained clinical significance, now forming an integral part of neuro-otological workup (Weber and Rosengren, 2015).

The vibration bursts used for bone-conducted oVEMP allow for stimulation at very high repetition rates. Based on this unique property, repetitive oVEMP stimulation represents an ideal examination technique for detecting a decrement in extraocular muscles. In a previous proof-of-concept study with 3, 10 and 20 Hz repetition rates, 20 Hz stimulation yielded the most effective results. The authors reached a sensitivity of 89% and a specificity of 64% (optimal cut-off of $\geq$15.2%) when using a unilateral decrement (whenever at least one of the two eyes showed a decrement) and a sensitivity of 63% and a specificity of 100% (optimal cut-off of $\geq$20.4%) when using the bilateral decrement (whenever both eyes showed a decrement) (Valko et al., 2016).

The aim of the current study was to optimize the stimulation parameters and improve the setup for the diagnosis of ocular myasthenia using repetitive oVEMP stimulation. For this purpose, we compared stimulation trains of 20 Hz, 30 Hz, 40 Hz and 50 Hz, 100 Hz continuous stimulation, as well as recordings from the inferior oblique and lateral rectus extraocular muscles in order to explore additional or alternate eye muscles for decrement detection.

2. Methods

This study was conducted at the Interdisciplinary Center for Vertigo and Neurological Visual Disorders, University Hospital Zurich, Switzerland. All participants gave their written informed consent according to the Declaration of Helsinki. The local ethics committee (Kantonale Ethik-Kommission Zurich, BASEC-Nr. 2016-01109) approved the study, which was registered under ClinicalTrials.gov (NCT03049956).

2.1. Study participants

18 myasthenia patients and 20 healthy volunteers without a history of vestibular or neurological disease participated in the current case-control study. The diagnosis of myasthenia was confirmed by the combination of a thorough clinical examination, serologic, edrophonium and/or electrophysiological (repetitive nerve stimulation of facial or accessory nerves, stimulated SFEMG of orbicularis oculi muscles) testing (Benatar, 2006, Barber, 2017). A number of patients underwent stimulated SFEMG with disposable single fiber EMG needles (25 $\times$ 0.45 mm, SEI EMG s.r.l., Citadella, Italy) using the SFEMG module of a VikingSelect EMG system (Natus Medical Inc. Pleasanton CA, USA). We defined abnormality of the jitter study according to Stalberg et al., 2016, with either a mean consecutive difference (MCD) value of >27 μs, or the detection of >2 outliers (MCD > 36 μs) (Stalberg et al., 2016). Myasthenia patients regularly under oral pyridostigmine treatment interrupted its intake overnight (for at least 12 h) prior to measurement.

2.2. Setup and recording parameters

As in the proof-of-concept study, participants were tested in supine position with their head elevated by a pillow (Valko et al., 2016). A red target on the ceiling marked the desired gaze direction, aiming at maximal upgaze.

In order to elicit a response decrement, we delivered 30 repetitions of ten bone-conducted vibration bursts per trial to the forehead. A hand-held mini-shaker (4810, amplifier 2706; Bruel and Kjaer, Naerum, Denmark) shielded with a μ-metal encasement was placed on the forehead at the hairline in the midline (the skull location identified as standard AFz' (Oostenveld and Praamstra, 2001, Iwasaki et al., 2008)). Customized software with a laboratory interface (Signal, version 5.02a; Cambridge Electronic Design (CED), Cambridge, UK) was used to generate the following stimuli with its built-in stimulus generator: vibration bursts of 4 ms at 500 Hz at four different repetition rates (20 Hz, 30 Hz, 40 Hz, 50 Hz), as well as 100 Hz continuous stimulation for a duration of 300 ms. The RMS (root mean square) of the voltage to drive the mini-shaker was 17 V (48 V peak-to-peak). These stimuli produced a predominant intermastoid head acceleration with an initial peak amplitude of 0.1 g in a different group of subjects of a previous experiment (Weber et al., 2012). After cleansing the skin with abrasive gel (Nuprep; Weaver and Company, Aurora, CO), surface electrodes (Blue Sensor NF; Ambu, Ballerup, Denmark) were mounted at the infraorbital margins to test inferior oblique muscles, with the reference electrodes directly below (similar to the regular oVEMP montage (Rosengren et al., 2010, Rosengren et al., 2013)). Additional electrodes were placed at the lateral canthus with references 1 cm inferiorly and laterally to test lateral rectus muscles (Fig. 1). Earth electrodes were placed on the chin. Recording was done with a power 1401 data acquisition device and a 1902 preamplifier system (CED). The signals were sampled at 10 kHz, bandpass filtered (5–2,000 Hz) and displayed with the previously mentioned software (Signal, version 5.02a; CED).

2.3. Experimental design

All oVEMP measurements were undertaken during one visit (lasting about 60 min) by the same examiner in the same room. A break of at least 2 min separated the five different sets of stimulation, to allow for recovery of eye muscle fatigue. We randomized the order of the applied stimulation paradigms using an open access randomization tool Urbaniak and Plous (2013). One set of stimulation consisted of 30 trains. Short pauses of 5 seconds partitioned each train, during which participants closed their eyes for 3–4 seconds (verbally paced by the examiner). A control measurement for electrical artefacts finalized the visit. For the artifact control measurement, a small amount of electrode gel provided electrical, but no mechanical contact between mini-shaker and skin. Electrodes were left in place during the entire measurement.

2.4. Data analysis and processing

Data analysis was performed using MatLab (version R2016b, 64-bit, MathWorks, Natick, MA, USA). We applied a 50 Hz notch filter (Q factor = 10) for rejection of mains noise, a 4th-order Butterworth high-pass filter (20 Hz cutoff frequency) for filtering blink artifacts and used a Median Absolute Deviation (MAD) algorithm

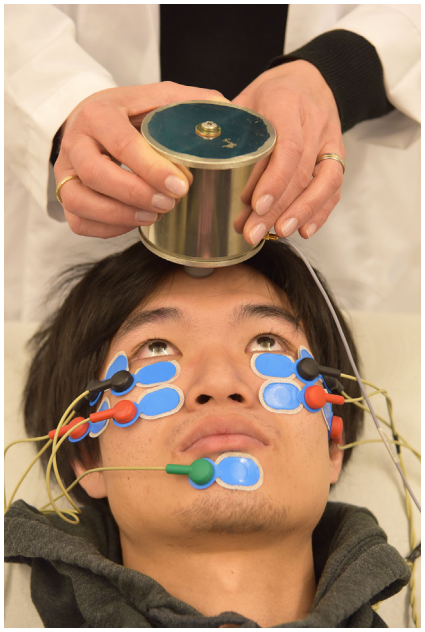

Fig. 1. Experimental setup of repetitive oVEMP stimulation. The mini-shaker for delivering bone-conducted vibration to the skull is placed near the hairline at midline (standard AFz' position). The subject is prompted to hold maximal upgaze during stimulation and relax in between. Responses from inferior oblique and lateral rectus extraocular muscles are recorded using surface electrodes (black: active, red: reference, green: grounding).

for automated outlier rejection. Prior to analysis, we averaged the 30 consecutive repetitions. Specific time frames for automated peak detection were set and the largest peak and trough within the preset timeframe was automatically selected by a custom software algorithm. Subsequently, the position of each peak and trough was verified visually and adjusted, if necessary. We used two different decrement calculation methods: in the 20 and 30 Hz paradigms, the amplitude of the potential was calculated from peak to trough within the preset second timeframe, and in the 40 and 50 Hz paradigms from the trough within the first timeframe to the peak within the second timeframe. This was necessary because due to shorter inter-stimulus intervals, the trough within the second timeframe was no longer discernible at higher repetition rates (40 and 50 Hz). In cases where the waves within a timeframe were biphasic, we used the largest amplitude for decrement calculation. All amplitudes of a train (responses one to nine) were measured. The decrement was defined as the ratio between the mean of the first two responses compared to the 6th-9th responses in the train, expressed as a percentage. To determine optimal decrement cutoff values and to visualize sensitivity and specificity, we generated receiver operating characteristic curves (ROC). Unilateral decrement curves considered the larger decrement of both eyes, bilateral decrement curves considered the smaller decrement of both eyes for analysis (Valko et al., 2016). To assess potential differences between the applied repetition rates we ran analyses of variance (two-way ANOVA) using different dependent variables (mean of left/right eye, maximum of left/right eye, minimum of left/right eye, difference right-left). In all cases, the factors were the type of subject (control versus patient) and the stimulus repetition rate (20 Hz, 30 Hz, 40 Hz, 50 Hz). For the analysis of 100 Hz continuous stimulation, moving averages based on rectified signals were calculated and used to generate envelope curves, illustrating means and 95% confidence intervals. Values are reported in the text and table as mean ± standard deviation.

3. Results

3.1. Participant characteristics

18 myasthenia patients and 20 healthy volunteers without a history of vestibular or neurological disease participated in the current study. Average ages of MG patients and healthy subjects were 62 ± 18 and 33 ± 10 years, respectively. Female to male ratios were 6:12 of MG patients and 10:10 of healthy controls. The average time from MG diagnosis to measurement amounted to 42 ± 70 months. The majority (89%) of MG patients had ocular symptoms at the time of measurement, including ptosis (n = 14) and diplopia (n = 12). Eight patients (44%) reported isolated ocular symptoms, four (22%) had additional bulbar weakness and nine (50%) generalized muscle weakness. AChR- and Titin-Ab were positive in 9/18 (50%) and 4/18 (22%) patients, respectively. MuSK-Ab were negative in all 18 patients. LRP-4- Ab were negative in 10 tested patients. Edrophonium testing was positive in 12 out of 13 tested patients, with one equivocal result. Results of electrophysiological assessments revealed a pathologic decrement of facial and/or accessory nerves in 8/13 (62%) patients and SFEMG of orbicularis oculi muscles showed a significant jitter in 5/6 (83%) patients (Table 1). Fourteen patients were on treatment at the time of examination (oral pyridostigmine [interrupted overnight before testing]: 14/18, oral prednisone: 3/18, oral azathioprine: 3/18). One patient previously underwent plasma exchange, another had received intravenous immunoglobulin. Three patients were newly diagnosed and one had stopped treatment voluntarily.

Table 1
Demographic, clinical and diagnostic patient characteristics of 18 myasthenia gravis patients.

Age (years)	62 ± 18				
Female	6 (18)				
Disease duration (months)	42 ± 70	oVEMP decrement (% mean both eyes), % sensitivity value			
Symptoms		20 Hz	30 Hz	40 Hz	50 Hz
All	18 (18)	−26.8%, 83.3%	−32.2%, 88.8%	−19.4%, 72.2%	−26.8%, 83.3%
Ptosis	14 (18)	−25.0%, 80%	−28.1%, 86.6%	−16.1%, 60.0%	−21.9%, 73.3%
Diplopia	12 (18)	−26.6%, 77%	−28.2%, 84.6%	−18.7%, 61.5%	−21.4%, 69.2%
Isolated ocular	8 (18)	−22.9%, 75%	−37.6%, 87.5%	−18.8%, 75.0%	−21.0%, 75.0%
Generalized	9 (18)	−26.8%, 88.8%	−25.1%, 88.8%	−24.6%, 77.7%	−19.6%, 66.6%
Bulbar	4 (18)	−34.4%, 100%	−37.1%, 100%	−8.7%, 66.6%	−27.5%, 80.0%
Antibody assays					
AchR-ab pos.	9 (18)	−27.7%, 88.9%	−29.9%, 88.9%	−24.3%, 66.6%	−22.9%, 66.6%
Titin-ab pos.	4 (18)	−15.6%, 80.0%	−20.2%, 100%	−7.3%, 66.6%	−11.2%, 66.6%
MusK-ab pos.	0 (17)	n/a	n/a	n/a	n/a
Anti-LRP4 pos.	0 (10)	n/a	n/a	n/a	n/a
Repetitive nerve stimulation (N VII/N IX) with decremental response	8 (13)	−20.7%, 75.0%	−19.6%, 87.5%	−15.9%, 62.5%	−26.7%, 75%
Edrophonium test pos.	12 (13)	−26.9%, 83.3%	−28.0%, 83.3%	−20.7%, 66.6%	−25.2%, 66.6%
Single-fiber-EMG (orbicularis oculi) with pos. jitter	5 (6)	−10.9%, 40.0%	−32.4%, 80.0%	−1.8%, 20.0%	−18.9%, 60.0%

3.2. Repetitive oVEMP stimulation

OVEMP responses, consisting of a series of peaks and troughs, were present in all study participants. Applying higher stimulus repetition rates resulted in an increasing overlap of oVEMP responses with subsequent stimulus artifacts. This wrap-around effect impeded the analysis of higher stimulus repetition rates to a certain extent. Fig. 2 shows oVEMP responses of a typical patient, using repetitive 20 Hz, 30 Hz, 40 Hz and 50 Hz stimulation, versus a normal subject tested with 30 Hz repetitive stimulation. Due to excessive blink artifacts and small amplitudes, we excluded the oVEMP results of two healthy controls and one MG patient from the analysis. None of the tested participants requested the measurement to be stopped.

3.2.1. Inferior oblique muscle recordings

Recordings from the inferior oblique muscles showed that oVEMP peaks became smaller (showing a decrement) over a train of stimuli in patients with myasthenia using all repetition rates. We found decrements of −27% in MG versus −4% in healthy

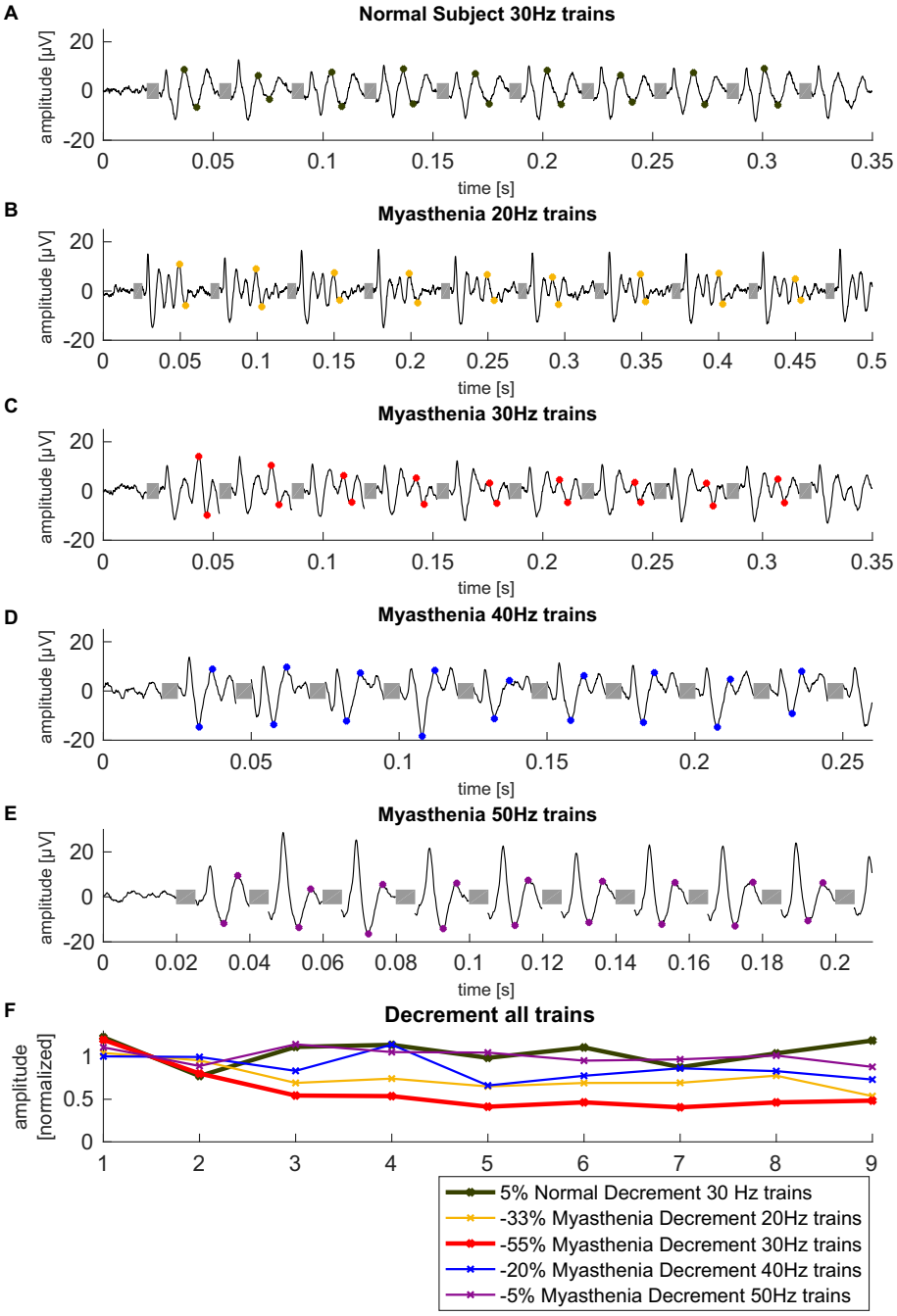

Fig. 2. Single subject data with decrement comparison at different stimulus repetition rates. Panel A shows repetitive oVEMP responses of a normal subject examined with stimulus trains of 30 Hz. Panels B–E demonstrate traces of a myasthenia patient using 20 Hz, 30 Hz, 40 Hz and 50 Hz repetitive oVEMP stimulation. Markers (asterisks) indicate peak to trough amplitudes that were used for decrement calculation. Panel F presents the juxtaposition of decremental/incremental responses of a normal subject (green 30 Hz) versus a myasthenia patient (orange 20 Hz, red 30 Hz, blue 40 Hz, violet 50 Hz). The magnitude of the decrement was calculated as the difference between the mean amplitude of the first and second stimulations and the mean amplitude of the sixth to ninth stimulations. Panels A–E: stimulus artefacts have been greyed out for clarity. OVEMP signals have been averaged from 30 consecutive trains.

controls using 20 Hz trains, −32% versus +18% (increment) using 30 Hz trains, −21% versus +12% using 40 Hz, −24% versus +7% using 50 Hz repetitive stimulation. At all repetition rates, the difference between controls and patients was statistically significant, as tested with a two-sample t-test ($p < 0.001$) (Table 2). For the two-way ANOVA of the mean of the right and left eyes, there was a significant main effect of subject type [$F(1,134) = 41408$, $p < 0.001$], but not of repetition rate [$F(3,134) = 0.99$, $p = 0.399$]. Further post-hoc testing (multiple comparison test), however, demonstrated a trend toward superiority of the 30 Hz repetition rate.

With higher repetition rates, a small increment appeared in healthy controls (18% with 30 Hz, 12% with 40 Hz, 7% with 50 Hz). Furthermore, with higher repetition rates, the decrement occurred after later stimuli in the train, i.e. using 20 Hz and 30 Hz trains, it occurred after the second stimulus, using 40 Hz trains after the third stimulus, using 50 Hz trains after the fourth stimulus (Fig. 3). Some outliers, with regard to decremental/incremental response, arose in the normal subjects due to small amplitudes, double peaks, and subtle blink artifacts. They evaded automatic outlier rejection and were therefore included in the analysis (Fig. 4, panels A–C).

3.2.1.1. ROC statistics of repetitive oVEMP stimulation from inferior oblique muscles. ROC analysis of repetitive stimulation with 30 Hz from recordings of the inferior oblique muscles scantly yielded the largest areas under the curve (Fig. 4). When including all eyes for the decrement analysis, sensitivity reached 76% and specificity 86% at a cut-off level of −9% (area under the curve (AUC) 0.83). Using a unilateral decrement (considering the eye with the larger decrement) and a cutoff of −9%, sensitivity amounted to 82%, specificity to 78% (AUC 0.81). Using a bilateral decrement (considering the eye with the smaller decrement) and −10% as cutoff, sensitivity values decreased to 71%, but specificity values increased to 94% (AUC 0.88). Detailed results of all stimulation parameters including 50 Hz (AUC all 0.78, unilateral 0.84, bilateral 0.76), 40 Hz (AUC all 0.79, unilateral 0.78, bilateral 0.83) and 20 Hz (AUC all 0.76, unilateral 0.84, bilateral 0.72) are listed in Table 3.

3.2.1.2. 100 Hz continuous stimulation at inferior oblique muscles. 100 Hz continuous stimulation could not discern MG patients from healthy controls. Mean decrement of normal subjects amounted to −7.9% ± 13.4% versus −7.2% ± 15.8% in MG patients ($p = 0.840$) (Fig. 5). However, the mean amplitude was higher in healthy controls than in MG patients (Fig. 5C).

3.2.2. Lateral rectus muscle recordings

Recordings from lateral rectus muscles also revealed decrements in MG patients. Our results, however, only allowed for significant separation between patients and controls at 30 Hz and 40 Hz. Mean decrements at 20 Hz were −17% in MG patients versus −9% in healthy controls ($p = 0.222$), −32% versus −12% at 30 Hz ($p = 0.027$), −2 % versus 7 % at 40 Hz ($p = 0.031$) and −10% versus 29% at 50 Hz ($p = 0.104$). (Table 2)

3.2.2.1. ROC statistics of repetitive stimulation from lateral rectus muscles. ROC analysis of repetitive stimulation from the lateral rectus muscles resulted in low sensitivity and specificity overall. Areas under the curve were largest using 50 Hz trains repetitive stimulation. Thus, considering all eyes and a cutoff level of −9% sensitivity of 56% and specificity of 64% was reached (AUC 0.67). Using a unilateral decrement and −10% as cutoff level, a sensitivity of 75% and a specificity of 50% was reached (AUC 0.76). Using the bilateral decrement and a cutoff of −9%, sensitivity decreased to 38%, and specificity increased to 78% (AUC 0.63). 50 Hz repetitive stimulation results were followed by 30 Hz (AUC all 0.62, unilateral 0.66, bilateral 0.58), 20 Hz (AUC all 0.56, unilateral 0.59, bilateral 0.56) and 40 Hz (AUC all 0.56, unilateral 0.68, bilateral 0.46) paradigms. See Table 3 for detailed results of all stimulation parameters with recordings from lateral rectus muscles (20 Hz, 30 Hz, 40 Hz, 50 Hz trains).

3.2.2.2. 100 Hz continuous stimulation at lateral rectus muscles. Similar to recordings from inferior oblique muscles, differentiation of MG patients and healthy controls was not possible with 100 Hz continuous stimulation and recordings from lateral rectus muscles.

Table 2
Detailed results of repetitive oVEMP stimulation.

Muscle	Stimulation Parameter		Mean Decrement ± Standard Deviation	Significance p (two-sided t-test)
Inferior oblique muscle	20 Hz trains	Normal	−4 ± 17%	<0.001
		Myasthenia	−27% ± 28%	
	30 Hz trains	Normal	+18% ± 47%	<0.001
		Myasthenia	−32% ± 34%	
	40 Hz trains	Normal	+12% ± 34%	<0.001
		Myasthenia	−19% ± 33%	
	50 Hz trains	Normal	+7% ± 35%	<0.001
		Myasthenia	−24% ± 34%	
Lateral rectus muscle	20 Hz trains	Normal	− 9% ± 31%	0.222
		Myasthenia	−17% ± 25%	
	30 Hz trains	Normal	−12% ± 39%	0.027
		Myasthenia	−32% ± 37%	
	40 Hz trains	Normal	+7% ± 27%	0.031
		Myasthenia	−2% ± 48%	
	50 Hz trains	Normal	+29% ± 137%	0.104
		Myasthenia	−10% ± 33%	

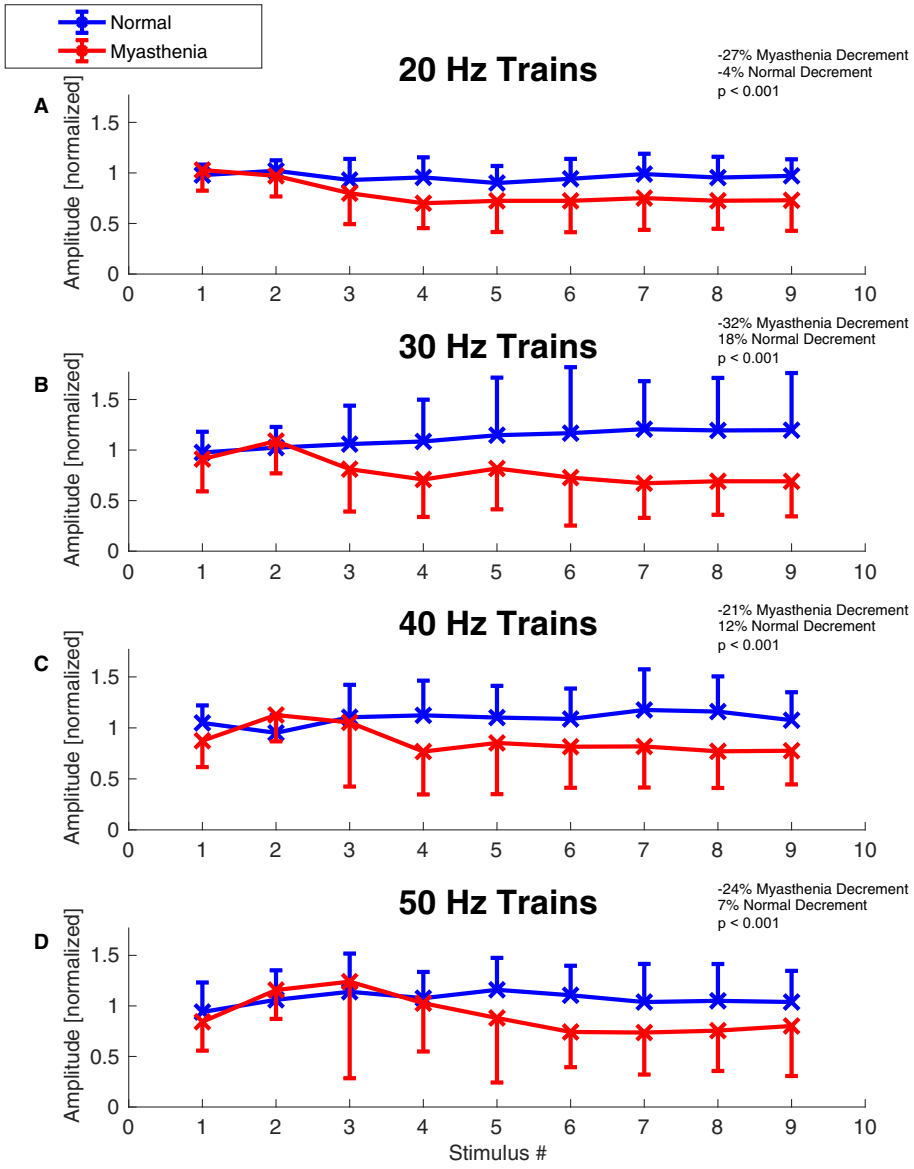

Fig. 3. Group comparison between patients with myasthenia gravis (MG) and healthy controls at four different stimulus repetition rates. Mean oVEMP responses of the first nine stimulations are shown for each paradigm. Each oVEMP response is indicated as normalized value ± SD with respect to the mean of the first and second stimulation as reference. All repetitive stimulation paradigms allowed reliable differentiation ($p < 0.001$) between MG patients and healthy controls.

100 Hz continuous stimulation led to a decrement of −2.6% ± 16.5% in normal subjects, and a decrement of −1.4% ± 16.0% in MG patients ($p = 0.755$).

3.3. Blink artifacts

A Median Absolute Deviation (MAD) algorithm automatically rejected outliers, which were typically due to blink artifacts. Rejection rate was highest with 40 Hz trains (mean rejection rate 15% ± 14%), yet similarly low with 20 Hz trains (11% ± 8%), 30 Hz trains (12% ± 8%), 50 Hz trains (11% ± 9%) and 100 Hz continuous stimulation (12% ± 8%). Mean rejection rates were similar in healthy controls and patients (7% ± 3% versus 11% ± 5%).

4. Discussion

This case-control study compared different repetitive oVEMP stimulation parameters for the diagnosis of MG. As recently described by Valko et al., repetitive oVEMP stimulation represents a novel, non-invasive diagnostic tool for ocular MG (Valko et al., 2016). Its major advantage, in comparison to standard diagnostic procedures, is the capability of directly targeting and measuring the extraocular muscles (EOM). This is crucial, because in early stages of the disease, fatigability is mostly constrained to EOMs (Wong et al., 2014). In the previous study (Valko et al., 2016), repetitive 20 Hz oVEMP stimulation yielded equally high sensitivity and specificity in isolated ocular MG versus generalized MG, and was more effective than 3 Hz and 10 Hz paradigms.

In the current study, we demonstrated that repetitive oVEMP stimulation of the inferior oblique muscles reliably differentiates MG patients from normal subjects at repetition rates between 20 Hz and 50 Hz, with a probable optimum at 30 Hz. Recording oVEMPs from the lateral rectus muscles did not improve our ability to differentiate between MG and controls and applying 100 Hz continuous stimulation for 300 ms was not useful either.

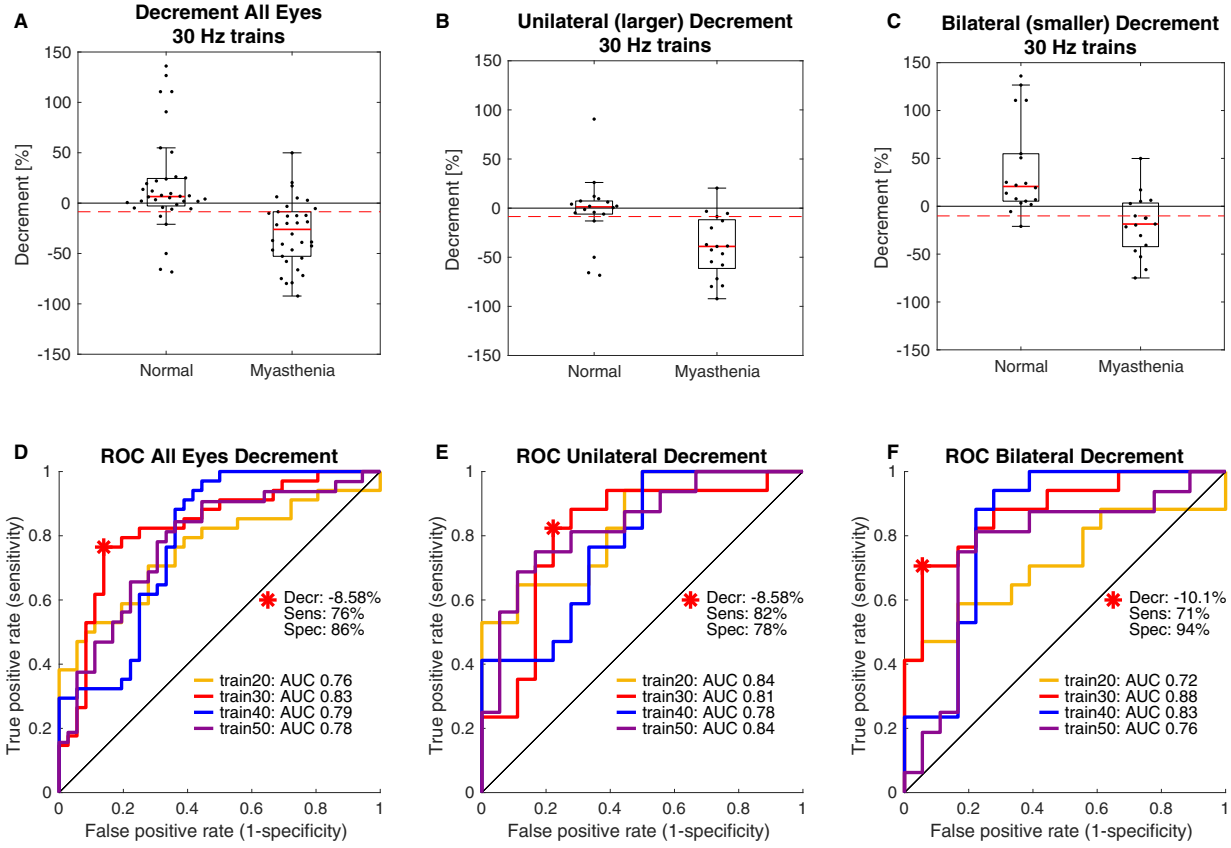

Fig. 4. Receiver operating characteristic curve (ROC) statistics for optimal cut-off determination. Box plots in panels A–C compare the distribution of participants and show data of the 30 Hz paradigm. The dashed red lines indicate the optimal diagnostic thresholds for all eyes (panel A), for eyes with the larger decrement, i.e. unilateral (panel B) and for eyes with the smaller decrement, i.e. bilateral (panel C), as derived from the red ROC curves shown in panels D–F. The boxes demonstrate median (red line) as well as first and third quartiles, while the ends of the whiskers represent the most extreme data points. Outliers are illustrated with black dots. Panels D–F show ROC curves and optimal cut-off levels (asterisks) for the 30 Hz paradigm. Area under the curve (AUC) was largest using 30 Hz trains (red) including all eyes and bilateral decrement (AUC all: 0.83, AUC bilateral: 0.88). At 30 Hz, a unilateral decrement of ≥9% had the advantage of high sensitivity of 82%, while a bilateral decrement of ≥10% resulted in excellent specificity values of 94%.

Table 3
Receiver operating characteristic (ROC) analyses.

Muscle	Stimulation Parameter	Decrement Analysis	Optimal cut-off	Sensitivity	Specificity	Area under the ROC curve
Inferior oblique muscle	20 Hz trains	All eyes	−15%	71%	72%	0.76
		Unilateral	−9%	94%	56%	0.84
		Bilateral	−16%	59%	83%	0.72
	30 Hz trains	All eyes	−9%	76%	86%	0.83
		Unilateral	−9%	82%	78%	0.81
		Bilateral	−10%	71%	94%	0.88
	40 Hz trains	All eyes	−7%	61%	75%	0.79
		Unilateral	−7%	70%	67%	0.78
		Bilateral	−7%	29%	83%	0.83
	50 Hz trains	All eyes	−5%	81%	67%	0.78
		Unilateral	−8%	88%	56%	0.84
		Bilateral	−5%	75%	83%	0.76
Lateral rectus muscle	20 Hz trains	All eyes	−10%	68%	53%	0.56
		Unilateral	−25%	71%	56%	0.59
		Bilateral	−12%	47%	72%	0.56
	30 Hz trains	All eyes	−9%	76%	47%	0.62
		Unilateral	−14%	100%	39%	0.66
		Bilateral	−9%	53%	56%	0.58
	40 Hz trains	All eyes	−27%	32%	92%	0.56
		Unilateral	−21%	59%	83%	0.68
		Bilateral	−20%	18%	89%	0.46
	50 Hz trains	All eyes	−9%	56%	64%	0.67
		Unilateral	−10%	75%	50%	0.76
		Bilateral	−9%	38%	78%	0.63

Unilateral Decrement Analysis = Larger decrement of both eyes is considered for decrement calculation, resulting in higher sensitivity values.
Bilateral Decrement Analysis = Smaller decrement of both eyes is considered for decrement calculation, resulting in higher specificity values.

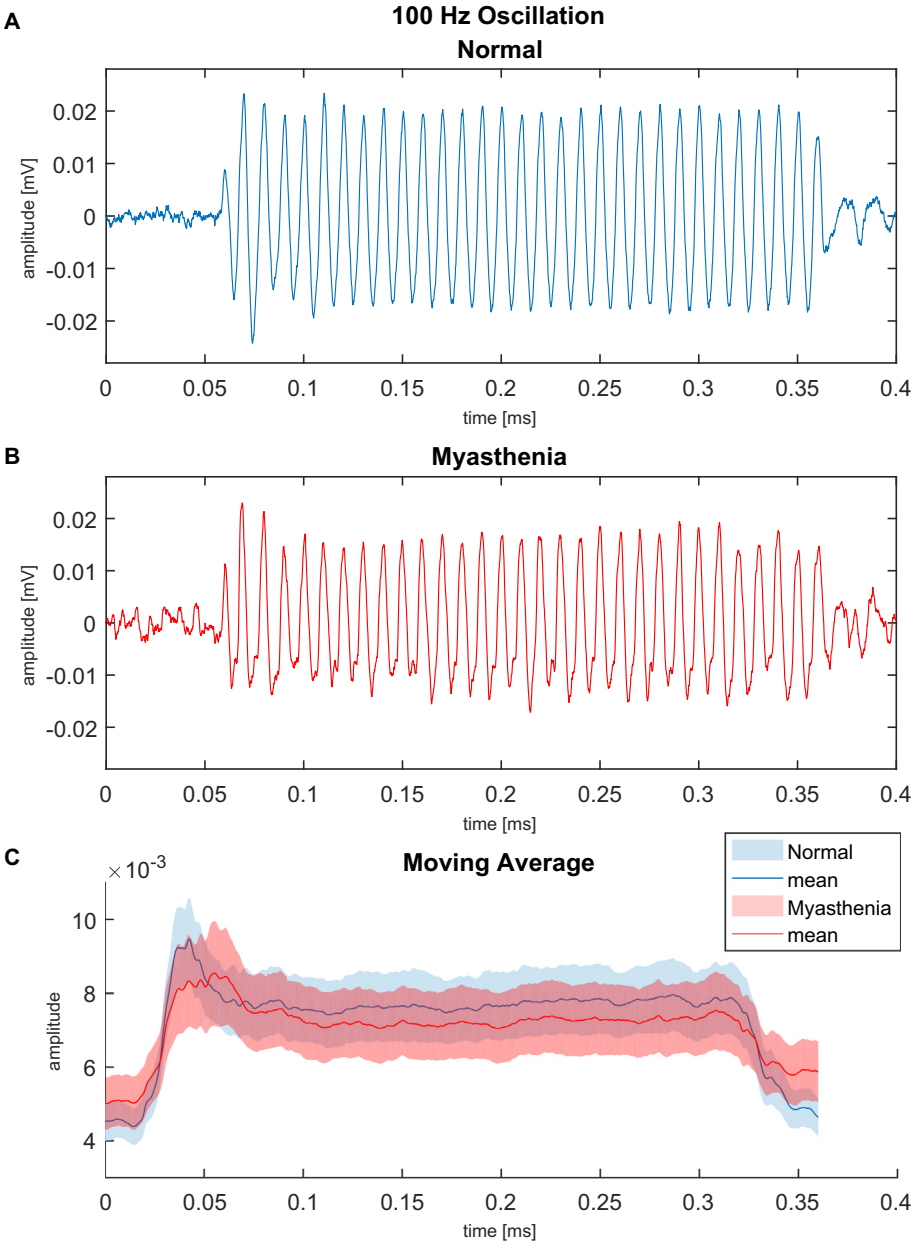

Fig. 5. 100 Hz continuous stimulation in normal subjects versus myasthenia patients. Panels A and B show examples of a normal control versus a myasthenia patient. As a group, mean decrements in myasthenia patients (red) did not significantly differ ($p = 0.755$) from normal subjects (blue). Panel C illustrates the envelopes of the responses, calculated as moving averages of the rectified signal over a full stimulation cycle (100 Hz). Means of moving averages over the stimulus duration (300 ms) are depicted as blue (controls) and red (patients) lines, bounded (transparent envelope) by respective 95% confidence intervals for the mean.

Higher repetition rates may have more power to unmask a decrement in (even sub-clinically) affected eyes. Higher repetition rates, however, concur with the mentioned wraparound effect, i.e. superimposition of oVEMP responses and subsequent stimulus artifacts, adversely affecting the clear differentiation of all peaks and troughs. This may have led to the non-superiority of the 40 and 50 Hz paradigms. We also observed that with higher repetition rates, the divergence of decremental/incremental responses occurred after a higher number of stimulus repetitions. Correspondingly, the decrement appears to be time-dependent. According to our data, repetition rates > 20 Hz seem to represent ideal stimulation parameters for the detection of a decrement in the uniquely structured extraocular muscles. As evident from the literature, human extraocular muscles feature particular characteristics and differ from limb skeletal muscles in several aspects (Porter et al., 1995, Bruenech and Kjellevold Haugen, 2015). Their specific morphology can be interpreted in the light of the particular demand in the ocular motor plant. Small motor units with 1:1 innervation, or even multiply innervated fibers, as well as uniquely structured muscle spindles, allow for fine-tuned contractions necessary for ocular stability and precise alignment (Scott and Collins, 1973, Wicke et al., 2007). Under physiologic conditions, effective neuromuscular transmission is characterized by a high *safety factor*, ensuring reliable transmission, despite slight changes of muscular environment (Waud and Waud,1975). Because of multiple immunological mechanisms, this *safety factor* is reduced to a variable extent in MG patients. The higher the repetition rates, the more effective the safety margin of neuromuscular transmission

is pushed to its limits. This explains the superiority of faster repetition rates in detecting a decrement in extraocular muscle reflex amplitude. Repetitive oVEMP stimulation allows for considerably higher rates, as compared to repetitive nerve stimulation in peripheral muscles (usually 2–3 Hz). The stimulus is not painful (as electrical stimulation can be), poses no harm to the cochlea (as air-conducted oVEMP stimulus can) and is associated with very little stimulus artefact. In addition to increasingly effective decrement responses, higher repetition rates entail the advantage of shortened testing duration.

In this study, age significantly differed between controls and patients. Even though it was reported, that oVEMP amplitudes could be affected by age (Piker et al., 2013), the current methodology involves measuring a decrement *within subjects* between two groups of stimuli, thus age differences are probably of minor relevance. However, this should be mentioned as a limitation of the current study. Similarly, this accounts for the measured peaks, which were automatically selected and did not necessarily reflect the standard vestibular oVEMP measures. Given that there was no change in peak selection between stimulus repetitions (within a patient), this also has a minor impact on decrement calculation. Besides that, all of the peaks are most probably VOR-related (Rosengren, 2010).

In accordance with previous oVEMP studies, our results show that recordings from the inferior oblique muscles in upgaze result in higher amplitudes and are therefore more suitable for diagnostic purposes than other extraocular muscles (Rosengren et al., 2013). Even though we were able to record oVEMP responses and detect decrements from lateral rectus muscles, differentiation between MG patients and healthy controls was not as effective. Smaller amplitudes and longer latencies may account for this finding. According to Govender et al., mediolateral electrode positioning appears to be even superior to conventional medial montage in neutral gaze for inferior oblique muscle recordings (Govender et al., 2016). This may be of particular importance in certain disease states, e.g. if MG hinders maximal upgaze due to severe ophthalmoparesis. Future studies should specifically evaluate medial versus mediolateral electrode positioning at the inferior oblique muscle for decrement detection purposes. Evaluating a potential correlation between the severity of eye muscle weakness and the decremental response would also be of interest. This may elucidate whether the degree of vertical gaze affected the results. Some patients had restricted upgaze (due to severe involvement of extraocular muscles in MG), which is typically associated with smaller oVEMP amplitudes, while sub-clinically affected patients were more likely to achieve maximal upgaze.

Unfortunately, 100 Hz continuous stimulation was not associated with a significant diagnostic yield (Fig. 5). The marked superimposition of stimulus artifacts and concurrent responses, masking a potential decrement, may account for this fact.

Interestingly, we observed a small increment in healthy subjects with higher repetition rates (Fig. 3). An incremental response to repetitive stimulation has been explained by an impairment of presynaptic release of acetylcholine which appears as a result of colliding anti- and orthodrome activity (Besser et al., 1989), or physiologic *post-tetanic facilitation* that is believed to be caused by increased synchrony of muscle fiber action potentials after tetanic stimulation (Katirji, 2007). Fast repetition rates may result in a short-term and reversible impairment of presynaptic release of acetylcholine (ACh) or increased synchrony of muscle fiber action potentials in healthy subjects. In pathologic conditions, incremental responses have been described in Lambert Eaton myasthenic syndromes (LEMS), where high repetition rates greatly enhance Ca^{2+} influx, resulting in larger releases of vesicles, with consequently larger endplate potentials (Tim and Sanders, 1994, Medicine, 2001, Katirji, 2007).

5. Conclusions

Repetitive oVEMP stimulation for the diagnosis of ocular myasthenia has several merits: it is non-invasive, simple and of short duration. In this study we were able to demonstrate that repetitive oVEMP stimulation with recordings from the inferior oblique muscles is robust between 20 Hz and 50 Hz repetition rates, yielding high discriminatory power between MG and healthy controls. Post-hoc testing indicated a probable optimum at 30 Hz repetition rate.

Optimal stimulation and recording parameters facilitate early and accurate diagnosis of ocular MG with relevant impact on the individual disease course, rendering this diagnostic method even more attractive for implementation in clinical practice. Further studies are needed to validate the diagnostic accuracy of repetitive oVEMP stimulation in comparison to standard diagnostic tests for ocular MG.

Acknowledgements

This study was supported by the Swiss National Science Foundation [320030_166346] and the Uniscientia Stiftung, Vaduz, Liechtenstein.

Conflicts of interest

None.

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