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ARTICLE

# Muscle velocity recovery cycles as pharmacodynamic biomarker: Effects of mexiletine in a randomized double-blind placebo-controlled cross-over study

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**Abstract**

Measuring muscle velocity recovery cycles (MVRCs) is a method to obtain information on muscle cell excitability, independent of neuromuscular transmission. The goal was to validate MVRC as a pharmacodynamic (PD) biomarker for drugs targeting muscle excitability. As proof-of-concept, sensitivity of MVRC to detect effects of mexiletine, a voltage-gated sodium channel ($Na_v$) blocker, was assessed. In a randomized, double-blind, two-way crossover study, effects of a single pharmacologically active oral dose of 333 mg mexiletine was compared to placebo in 15 healthy male subjects. MVRC was performed predose, and 3- and 5-h postdose using QTrac. Effects of mexiletine versus placebo were calculated using a mixed effects model with baseline as covariate. Mexiletine had significant effects on MVRC when compared to placebo. Early supernormality after five conditioning stimuli was decreased by mexiletine (estimated difference −2.78% [95% confidence interval: −4.16, −1.40]; $p$ value = 0.0003). Moreover, mexiletine decreased the difference in late supernormality after five versus one conditioning stimuli (5XLSN; ED −1.46% [−2.26, −0.65]; $p = 0.001$). These results indicate that mexiletine decreases the percentage increase in velocity of the muscle fiber action potential after five conditioning stimuli, at long and short interstimulus intervals, which corresponds to a decrease in muscle membrane excitability. This is in line with the pharmacological activity of mexiletine, which leads to use-dependent $Na_V1.4$ blockade affecting muscle membrane potentials. This study shows that effects of mexiletine can be detected using MVRC in healthy subjects, thereby indicating that MVRC can be used as a tool to demonstrate PD effects of drugs targeting muscle excitability in early phase drug development.

**Study Highlights**

**WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?**

Muscle velocity recovery cycles (MVRC) is a method to obtain information on muscle excitability, independent of neuromuscular transmission. MVRC has been used to distinguish various neuromuscular diseases from healthy controls.

**WHAT QUESTION DID THIS STUDY ADDRESS?**

Can MVRC be a valuable pharmacodynamic (PD) biomarker for drugs targeting muscle excitability?

**WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?**

As proof-of-concept, we evaluated effects of mexiletine—a sodium channel blocker expected to decrease muscle excitability—on MVRC in a double-blind, placebo-controlled study in healthy subjects. We demonstrated significant effects of mexiletine on MVRC, indicating reduced muscle excitability, in line with the pharmacological mechanism of action.

**HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?**

Our results encourage use of MVRC as a tool to demonstrate PD effects in the development of drugs targeting muscle excitability. This biomarker may be used to demonstrate target engagement in early clinical drug development. Furthermore, it could be of interest as a biomarker in the translation from preclinical to clinical studies, and from healthy subjects to patients with neuromuscular disease.

## INTRODUCTION

Neuromuscular diseases (NMDs) have received growing attention in preclinical and clinical research in recent decades, which has led to increased understanding of these disorders. However, significant progress is still to be made where it comes to developing treatment options for these patients. An essential part of advancing treatments through (pre)clinical drug development toward therapy is the use of biomarkers, especially for these often complex disorders.[1] Such biomarkers should be tailored to specific NMDs, as they are a collection of rare disorders with a broad spectrum of underlying pathophysiology. However, despite their heterogeneity, a common feature for many of these diseases is direct or indirect muscle pathology, resulting in symptoms of muscle weakness and other muscle pathology. A biomarker that can characterize these defects and allows quantification of pharmacological effects, would therefore be of great value in drug development for a relevant subset of NMDs.

Muscle velocity recovery cycle (MVRC) measurements could be such a pharmacodynamic (PD) biomarker, as they evaluate muscle cell excitability in vivo and are considered to be independent of neuromuscular transmission.[2] The physiological muscle action potential is followed by early and late depolarizing afterpotentials, resulting in two periods of increased excitability. By applying one or more conditioning pulses before the test pulse, MVRC can indirectly quantify these afterpotentials as periods of increased velocity (supernormality).[2] Previous studies showed that MVRC was able to distinguish different types of NMD from healthy controls, indicating that the method has analytical and clinical validity. Abnormalities in MVRC endpoints were detected in critical illness neuropathy, Anderson Tawil syndrome, channelopathies, erythromelalgia, myotonic dystrophies, inclusion body myositis, hypo- and hyperkalemic periodic paralysis, sodium channel myotonias, and myotonia congenita.[3–12]

However, to our knowledge, sensitivity of MVRC to detect (acute) PD effects has not been evaluated. Therefore, the primary aim of this study was to investigate whether MVRC could detect pharmacologically induced changes in muscle excitability in healthy subjects. As a proof-of-concept, we selected mexiletine as pharmacological intervention. Mexiletine is a use-dependent voltage-gated sodium ($Na_V$) channel blocker, thought to influence muscle excitability through blocking $Na_V$ channels subtype 1.4 in skeletal muscle fibers.[13–15] As a secondary objective, this study was set up to evaluate the feasibility and repeatability of MVRC for use in an early phase clinical drug study.

## MATERIALS AND METHODS

This trial was approved by the Foundation "Beoordeling Ethiek Biomedisch Onderzoek," an independent ethics

committee based in Assen, The Netherlands. The trial was executed between January 2020 and March 2020, in accordance with the Declaration of Helsinki. The study was registered in the Dutch Trial Registry (Nederlands Trial Register, registration number NL8084).

## Study design

This was a randomized, double-blind, placebo-controlled, two-way cross-over study in healthy subjects. Subjects received a single dose of mexiletine 333 mg and matching placebo in randomized order on two separate study visits. Drug administrations were separated by a washout period of 7 days. MVRC measurements were performed predose and at two postdose timepoints based on the pharmacokinetic profile of mexiletine. The first postdose measurement was performed 3 h postdose (approximate time to maximum concentration), the second at 5 h postdose (another measurement at expected high plasma concentrations of mexiletine), maximizing the power to detect a PD effect. Measurement conditions and mealtimes were standardized, and measurements were performed at approximately the same clock time, to avoid interference of diurnal variation or effects of food. A follow-up visit was performed 5–9 days after the last dose administration.

No important changes were made to the methods or trial outcomes after study commencement.

## Study population

All subjects signed written informed consent before participation in the study. To confirm eligibility and health status, subjects were screened before participation, based on an interview of medical history, physical examination (including vital signs and electrocardiogram), and laboratory tests. Subjects were aged between 18 and 45 years, with a body mass index between 18 and 30 $kg/m^2$ and a minimum weight of 50 kg. Subjects with active or chronic disease that could interfere with the safety or conduct of the study were excluded, particularly a history of trauma to the lower extremities or other conditions that could interfere with the MVRC measurements. The use of medication, dietary supplements, CYP-enzyme containing products, alcohol, and caffeine were prohibited during the study. Subjects with a history of addictive substance abuse were excluded, and drug and alcohol tests were performed to determine current use of these substances. Excessive exercise was prohibited within 72 h before dosing.

## Study drugs, randomization, and blinding

Mexiletine (Namuscla, 167 mg; Lupin Europe GmbH) and matching placebo were administered as capsules. The matching placebo was indistinguishable from the active drug. A dose of 333 mg mexiletine was chosen as it was thought to be pharmacodynamically active, because the recommended therapeutic dose for patients with myotonia congenita is between 200 and 600 mg mexiletine hydrochloride daily (167–500 mg mexiletine). Moreover, a dose of 333 mg mexiletine was considered safe for healthy subjects—doses up to 600 mg mexiletine have been administered.[16]

The randomization schedule was generated using SAS version 9.4 (SAS Institute, Inc.) by an unblinded statistician, who was not involved in the clinical execution of the study. A balanced treatment allocation (2 sequences, each for 6 subjects) was chosen to control for first-order carryover effects. Blinded study staff enrolled subjects and assigned participants to interventions. All participants and study staff remained blinded during the study.

## Muscle velocity recovery cycles

Practical details of the MVRC procedure were described previously.[2,17] We performed the measurements in the distal tibialis anterior muscle. A monopolar needle electrode (Natus Dantec DCN, 25 mm × 26G) for stimulation was inserted ~1 cm proximal to the distal end of the muscle. The anode surface electrode (BlueSensor NF, Ambu) was placed distal to—and in near proximity of—the monopolar needle. A concentric recording needle electrode (25 mm × 30G, TECA elite; Natus) was placed 2 cm proximal to the monopolar electrode. Needles were inserted perpendicular to the skin, to a depth of ~1 cm. A ground electrode (Red dot; 3M) was placed on the medial malleolus. Stimulation was computer guided by QTracS software (protocol M3REC6; Institute of Neurology, London, UK). Pulses were applied by an isolated bipolar constant-current stimulator (DS5; Digitimer). The recordings were amplified (gain 1000, bandpass filter 3 Hz to 3 kHz) using an EMG amplifier (D440-2; DigiTimer). An analog-digital convertor (NI-USB-6341, National Instruments) digitized the signal at a sampling frequency of 20 kHz. Hum Bug (Quest Scientific Instruments) was used to minimize 50 Hz noise. Skin temperature was held between 32 and 36°C by an infrared lamp (Daylight Heat Lamp; General Electronic). Skin temperature was recorded at the beginning and end of the measurement.

Two stimulation paradigms were applied: recovery cycles with one, two, and five conditioning stimuli; and

frequency ramp. In the first paradigm, conditioning pulses are applied at interstimulus intervals (ISIs) of 10 ms. After the last conditioning pulse, a test pulse is applied at a decreasing ISI between 1000 and 1.8 ms in 33 steps: 1000, 900, 800, 700, 600, 500, 450, 400, 350, 300, 260, 220, 180, 140, 110, 89, 71, 56, 45, 35, 28, 22, 18, 14, 11, 8.9, 7.1, 5.6, 4.5, 3.5, 2.8, 2.2, and 1.8 ms. In the frequency ramp paradigm, a train of conditioning pulses is applied with a frequency ranging between 1 and 30 Hz.[11]

Moreover, 15-point repeated recovery cycles measurements before, during, and after 5 min of ischemia induced by a blood pressure cuff around the upper leg. Execution of this complex measurement proved challenging which led to limited data quality; therefore, it is not reported.

## Data handling

MVRC variables were generated using QTracP (Institute of Neurology, London, UK), details described previously.[2]

From the recovery cycles' recordings, latency from test stimulus to peak muscle action potential is measured. The effect of conditioning stimuli on the latency after the test pulse are estimated as the percentage change compared to an unconditioned test pulse.[8,11] As published previously,[11] the following end points were generated for recovery cycles with one, two, and five conditioning stimuli. Muscle relative refractory period (MRRP): interpolated ISI at which the latency of the unconditioned response and latency of the response after one conditioning stimulus are the same. Early supernormality (ESN): peak percentual latency change induced by one conditioning stimulus at ISIs less than 15 ms. Early supernormality is also calculated for five conditioning pulses: 5ESN. Time to peak ESN (ESN@) is the ISI corresponding to ESN. SN20 is the supernormality at ISI 20 ms. Late supernormality (LSN) is defined as the mean percentage latency change due to one conditioning stimulus, at ISIs between 50 and 150 ms. XLSN: the difference in LSN between two and one conditioning stimuli, and 5XLSN: the difference in LSN between five and one conditioning stimuli. Residual supernormality (RSN) is the percentage latency change between ISIs 900 and 1000 ms, and 5XRSN is the difference in RSN between five and one conditioning stimuli.

For the frequency ramp, latency change is calculated as the percentage of unconditioned action potentials recorded before the ramp.[11] Latency changes after stimulus trains with pulse frequencies of 15 Hz (Lat[15 Hz]) and 30 Hz (Lat[30 Hz]) were calculated, as well as percentage change in amplitudes of the action potentials after 15 Hz (Peak[15 Hz]) and 30 Hz (Peak[30 Hz]) trains. The minimal latency (expressed as percentage of the unconditioned pre-ramp potential) measured during the ramp is LatMin, the corresponding frequency when latency is minimal is FreqLatMin. Latency and amplitude changes are calculated for the first and last potential in each train, and these are indicated as "First" and "Last". Percentage change in amplitude between 30 and 15 Hz (Peak[30-15 Hz]) is calculated, as well as percentage latency and peak change 30 s after the ramp (Lat[30Hz30s] and Peak[30Hz30s], respectively).

Before generation of the end points, raw data was visually inspected by blinded study staff, and interpolation of single datapoints was performed in case of single outliers with an abnormal muscle response. Additionally, a blinded data review was performed to remove measurements with technical abnormalities from analysis.

## Statistical analysis

Statistical analysis was performed in SAS version 9.4 (SAS Institute, Inc.). Visual evaluation of normal distribution was performed during analysis, and no variables needed log-transformation to correct for log-normal distribution. Repeatedly measured MVRC data are analyzed with a mixed effects model with fixed factors: treatment, period, time and treatment by time, random factors: subject, subject by treatment and subject by time, and the average pre-value as covariate. The contrast calculated within the model is placebo versus mexiletine. To indicate inter- and intrasubject variability of MVRC, coefficients of variation (CV%) were calculated from placebo measurements (within-day variability) and derived from the raw data as well as model covariate variables. Statistical significance was defined at the 5% level.

We used previously published variability data of MVRC in healthy subjects[18] to estimate the required sample size. Because no PD effects on MVRC had been reported previously in healthy subjects, expected effect sizes for this study were based on those observed with ischemia.[2] A sample size of 12 subjects in a cross-over design would be able to detect a difference in MRRP of 0.37 ms, and difference in ESN of 1.16%. with a power of 0.8.

# RESULTS

A total of 15 subjects were enrolled, of which 14 subjects completed the study. This includes three replacement subjects enrolled due to insufficient quality of MVRC measurements in three of the first 12 subjects. Demographics are summarized in Table S1.

A total of 85 measurements were performed in 15 subjects. One subject only underwent two measurements

and was subsequently excluded. One measurement in another subject was not obtained for technical reasons. Additionally, the following measurements were excluded from analysis in a blinded data review (see chapter Data handling): for 11 measurements, the recovery cycles were (partially or fully) excluded, for eight measurements, the frequency ramp was (partially or fully) excluded.

Individual and mean plasma concentrations of mexiletine are shown in Figure S1, mean concentrations per protocol time are in Table S2. Adverse events reported in the study were mild to moderate in intensity, and transient.

## Test–retest reliability

Test–retest reliability, estimated in CV%s, of all MVRC variables is shown in Table S3. Raw baseline MVRC end points and estimated means of measurements 3- and 5-h postdose, are shown in Table S4.

## Effects of mexiletine on recovery cycles

Effects of mexiletine on recovery cycles are listed in Table 1. Mexiletine significantly decreased early supernormality after five conditioning stimuli (5ESN) compared to placebo (Figure 1). Moreover, difference in LSN after five versus one conditioning stimuli (5XLSN) was significantly decreased (Figure 2).

To visualize these treatment effects, average postdose recovery cycles recordings with five conditioning stimuli are shown in Figure 3 for mexiletine and placebo. Average postdose recovery cycles recordings with one conditioning stimulus and two conditioning stimuli are shown in Figures S2 and S3, respectively.

## Effects of mexiletine on frequency ramp

Effects of mexiletine versus placebo on frequency ramp are listed in Table 1. Mexiletine significantly increased the percentual latency after the last pulse of a 15 Hz train ($Lat[15\,Hz]_{last}$) and a 30 Hz train ($Lat[30\,Hz]_{last}$), as shown in Figures 4 and 5, respectively. Moreover, mexiletine increased the minimal latency during the ramp ($LatMin_{last}$) and decreased the frequency at which the latency was minimal ($FreqLatMin_{last}$; Figures S4 and S5, respectively).

Average postdose frequency ramp recordings (Figure 6) visualize these effects, showing that the latency decrease due to the 15 and 30 Hz trains is reduced by mexiletine.

# DISCUSSION

The aim of this study was to investigate the use of MVRC as a tool to demonstrate PD effects on muscle excitability. As a proof-of-concept, we compared effects of mexiletine to placebo in healthy subjects and were able to demonstrate significant effects of mexiletine on several MVRC variables. The recovery cycles variables 5ESN and 5XLSN were decreased by mexiletine, indicating that mexiletine decreases supernormality of the muscle action potential after five conditioning stimuli, at long and short ISIs. Moreover, we detected a significant increase of $Lat[15\,Hz]_{Last}$, $Lat[30\,Hz]_{Last}$, $LatMin_{last}$, and $FreqLatMin_{last}$ by mexiletine using the frequency ramp paradigm. In this paradigm, a train of conditioning stimuli physiologically results in an increase in latency at the end of the train—we show that mexiletine suppresses this latency increase after a 15 and 30 Hz stimulus train.

These results indicate that MVRC end points are sensitive to detect effects of pharmacological interventions on muscle excitability. The effects on 5ESN and 5XLSN, and $Lat[15\,Hz]_{Last,}$ $Lat[30\,Hz]_{Last}$, $LatMin_{last}$, and $FreqLatMin_{last}$, can be explained by the mechanism of action of mexiletine. Mexiletine reduces muscle cell excitability through a use-dependent block of $Na_V1.4$, with higher affinity for $Na_V$ channels in the open and inactivated state.[13–15] This pharmacological property may explain why mexiletine significantly reduces early and late supernormality after five conditioning pulses, as an increased number of $Na_V1.4$ channels will be in the open or inactivated state after previous activations shortly before the test pulse. Additionally, our finding that ESN is only affected by mexiletine after *five* conditioning stimuli, and not after one or two conditioning stimuli, may be explained by the use-dependence of the $Na_V$ blockade, as fewer conditioning stimuli would result in a relatively lower availability of inactivated $Na_V$ channels that can be bound by mexiletine. When observing effects of mexiletine on postdose recovery cycles recordings of one (Figure S2) and two conditioning stimuli (Figure S3), there is no effect on recovery cycles with one conditioning stimulus, and a small (nonsignificant) effect on supernormality after two conditioning stimuli, in the same direction as the effect seen with five conditioning stimuli (Figure 3). This appears to indicate that the effect of mexiletine indeed increases with an increasing number of conditioning stimuli. The effects on frequency ramp—significant decrease in supernormality due to stimulus trains at high frequencies ($Lat[15\,Hz]_{Last}$ and $Lat[30\,Hz]_{Last}$)—also corresponds to effects expected from a use-dependent $Na_V$ block: effects of mexiletine are larger after repetitive stimulation. Additionally, the difference between mexiletine and placebo is much

**TABLE 1** Effects of mexiletine versus placebo on MVRC end points, shown as the estimated mean of the treatment period (postdose) and the estimated difference of mexiletine versus placebo, reported with 95% confidence interval and *p* value

| | Treatment | Estimated mean treatment period | Estimated difference | 95% confidence interval | *p* Value |
|---|---|---|---|---|---|
| Recovery cycles with 1, 2, and 5 conditioning stimuli | | | | | |
| MRRP, ms | Placebo | 3.03 | | | |
| | Mexiletine | 3.09 | 0.058 | (−0.250, 0.365) | 0.702 |
| ESN, % | Placebo | 12.40 | | | |
| | Mexiletine | 11.55 | −0.854 | (−2.760, 1.051) | 0.328 |
| ESN@, ms | Placebo | 6.27 | | | |
| | Mexiletine | 6.62 | 0.34 | (−0.48, 1.17) | 0.401 |
| 5ESN, % | Placebo | 13.41 | | | |
| | Mexiletine | 10.64 | −2.78 | (−4.157, −1.396) | **<0.001*** |
| SN20, % | Placebo | 6.42 | | | |
| | Mexiletine | 5.92 | −0.497 | (−1.33, 0.340) | 0.230 |
| LSN, % | Placebo | 3.19 | | | |
| | Mexiletine | 3.26 | 0.075 | (−0.527, 0.676) | 0.797 |
| 2XLSN, % | Placebo | 2.47 | | | |
| | Mexiletine | 2.08 | −0.39 | (−0.811, 0.032) | 0.068 |
| 5XLSN, % | Placebo | 6.95 | | | |
| | Mexiletine | 5.49 | −1.46 | (−2.258, −0.653) | **0.001*** |
| RSN, % | Placebo | 0.166 | | | |
| | Mexiletine | 0.165 | −0.001 | (−0.331, 0.330) | 0.997 |
| 5XRSN, % | Placebo | 0.888 | | | |
| | Mexiletine | 0.717 | −0.171 | (−0.573, 0.231) | 0.388 |
| Frequency ramp | | | | | |
| $Lat[15\,Hz]_{first}$, % | Placebo | 96.3 | | | |
| | Mexiletine | 96.5 | 0.20 | (−0.69, 1.10) | 0.650 |
| $Lat[15\,Hz]_{last}$, % | Placebo | 86.6 | | | |
| | Mexiletine | 89.3 | 2.77 | (0.99, 4.55) | **0.004*** |
| $Lat[30\,Hz]_{first}$, % | Placebo | 97.2 | | | |
| | Mexiletine | 98.2 | 0.98 | (−0.75, 2.71) | 0.252 |
| $Lat[30\,Hz]_{last}$, % | Placebo | 87.4 | | | |
| | Mexiletine | 95.0 | 7.58 | (3.80, 11.4) | **<0.001*** |
| Lat[30 Hz + 30 s], % | Placebo | 101.6 | | | |
| | Mexiletine | 100.7 | −0.90 | (−2.30, 0.49) | 0.190 |
| $Peak[15\,Hz]_{first}$, % | Placebo | 110.5 | | | |
| | Mexiletine | 109.5 | −1.02 | (−9.24, 7.19) | 0.801 |
| $Peak[15\,Hz]_{last}$, % | Placebo | 107.5 | | | |
| | Mexiletine | 110.4 | 2.84 | (−12.45, 18.14) | 0.692 |
| $Peak[30\,Hz]_{first}$, % | Placebo | 112.8 | | | |
| | Mexiletine | 112.6 | −0.13 | (−13.48, 13.21) | 0.983 |
| $Peak[30\,Hz]_{last}$, % | Placebo | 88.3 | | | |
| | Mexiletine | 89.5 | 1.20 | (−19.45, 21.84) | 0.903 |
| Peak[30-15 Hz], % | Placebo | 1.80 | | | |
| | Mexiletine | 4.49 | 2.69 | (−3.49, 8.86) | 0.376 |

**TABLE 1** (Continued)

| | Treatment | Estimated mean treatment period | Estimated difference | 95% confidence interval | *p* Value |
|---|---|---|---|---|---|
| Peak[30 Hz + 30 s], % | Placebo | 98.1 | | | |
| | Mexiletine | 97.8 | −0.23 | (−7.28, 6.82) | 0.948 |
| $LatMin_{first}$, % | Placebo | 95.4 | | | |
| | Mexiletine | 95.9 | 0.45 | (−0.80, 1.70) | 0.435 |
| $LatMin_{last}$, % | Placebo | 85.01 | | | |
| | Mexiletine | 88.76 | 3.75 | (1.55, 5.95) | **0.002*** |
| $FreqLatMin_{first}$, Hz | Placebo | 20.12 | | | |
| | Mexiletine | 18.54 | −1.57 | (−5.48, 2.33) | 0.412 |
| $FreqLatMin_{last}$, Hz | Placebo | 21.61 | | | |
| | Mexiletine | 17.79 | −3.82 | (−6.09, −1.54) | **0.002*** |

*Note*: Significant results are highlighted with *.

Abbreviations: ESN, early supernormality; ESN@, time to peak early supernormality; LSN, late supernormality; MRRP, Muscle relative refractory period; MVRC, muscle velocity recovery cycle; RSN, Residual supernormality; SN20, supernormality at interstimulus interval 20 ms.

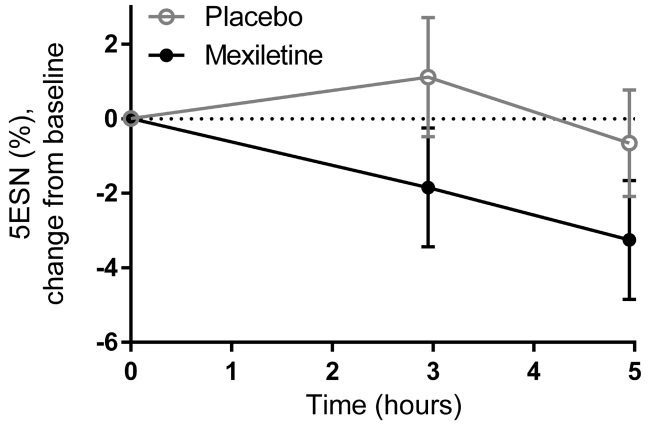


**FIGURE 1** Effects of mexiletine versus placebo on early supernormality after five conditioning stimuli (5ESN), shown as the estimated mean change from baseline at 3- and 5-h postdose. Error bars indicate the 95% confidence interval of the estimated mean.

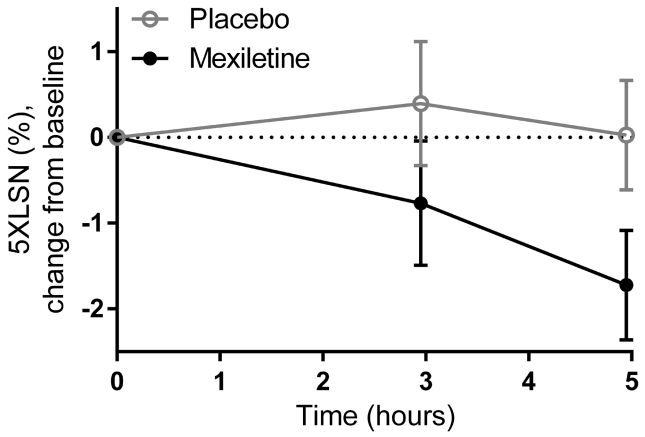


**FIGURE 2** Effects of mexiletine versus placebo on the difference in late supernormality of five versus one conditioning stimuli (5XLSN), shown as the estimated mean change from baseline at 3- and 5-h postdose. Error bars indicate the 95% confidence interval of the estimated mean.

larger after 30 Hz trains than 15 Hz trains, suggesting an increasing effect at higher stimulation frequencies.

To our knowledge, this is the first study to evaluate effects of $Na_V$ blockers on muscle excitability using MVRC in placebo-controlled manner. An interesting report in this context, however, evaluated effects of a gain-of-function mutation in $Na_V1.4$ channels on MVRC in patients with sodium channel myotonia.[9] This mutation results in slowed $Na_V$ inactivation,[9] which should theoretically exhibit somewhat opposite effects to mexiletine as $Na_V1.4$ blocker. Indeed, 5ESN and 5XLSN (among others) were significantly increased, and $Lat[15\,Hz]_{last}$ and $Lat[30\,Hz]_{last}$ significantly decreased in sodium channel myotonia, strengthening our results and confirming the mechanism involved in influencing MVRC.

Another relevant paper in this context describes muscle excitability in patients with myotonia congenita. Patients with myotonia congenita carry a mutation in ClC-1, resulting in an increase in muscle excitability. The authors compared MVRC of patients with myotonia congenita off-treatment, to patients using $Na_V$ blockers (mainly mexiletine).[11] Tan et al. showed that the presence of myotonia congenita (in patients who are not on treatment) results in an increase in ESN, 5ESN, LSN, and 5XLSN compared with healthy subjects. The authors showed that patients on-treatment with $Na_V$ blockers have a significant decrease in all these variables (a change in the direction of normal controls). This suggests a (partial) reversing of the effects of myotonia congenita by $Na_V$ blockers. Although the results

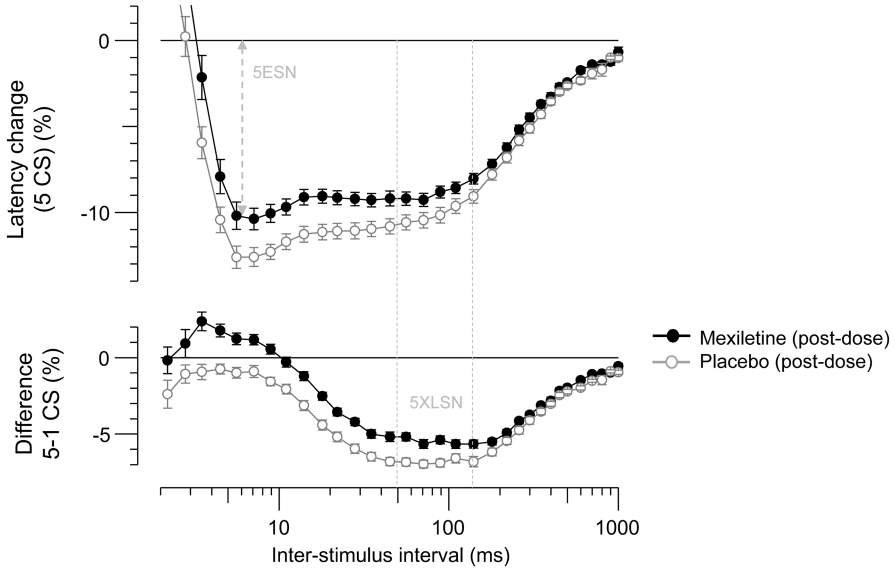


**FIGURE 3** Mean postdose recordings of recovery cycles with five conditioning stimuli (CS), for mexiletine (black, filled) and placebo (gray, empty). Error bars show the standard error. The upper graph shows the percentual latency change after five conditioning stimuli at different interstimulus intervals. The lower graph shows the additional change in latency of five versus one conditioning stimuli. Variables with significant effects (mexiletine vs. placebo) are visualized by indicating the name of the variable. Variable visualization is reproduced from ref. [11]. Note this graph is meant to visualize treatment effects, but does not fully reflect the statistical analysis, because the statistical model includes baseline as a covariate which is not reflected in the graph.

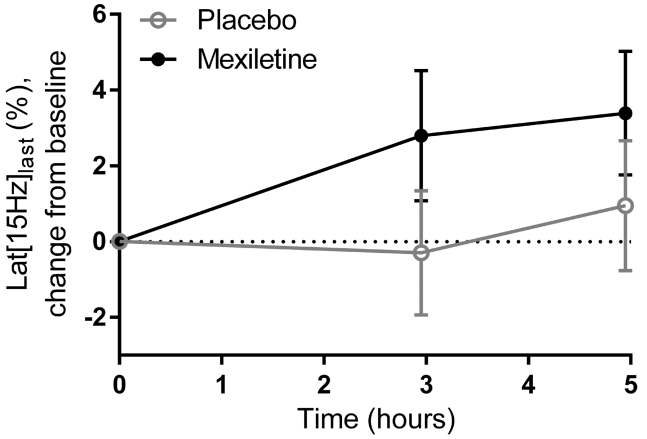


**FIGURE 4** Effects of mexiletine versus placebo on the latency change after a 15 Hz train of stimuli ($Lat[15\,Hz]_{last}$), shown as the estimated mean change from baseline at 3- and 5-h postdose. Error bars indicate the 95% confidence interval of the estimated mean.

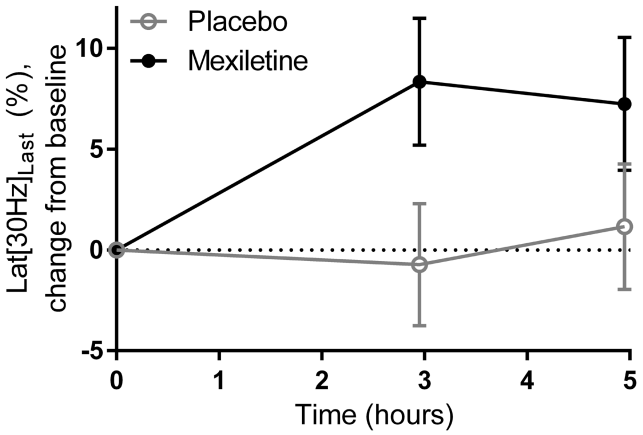


**FIGURE 5** Effects of mexiletine versus placebo on the latency change at the end of a 30 Hz train of stimuli ($Lat[30\,Hz]_{last}$), shown as the estimated mean change from baseline at 3- and 5-h postdose. Error bars indicate the 95% confidence interval of the estimated mean.

cannot directly be compared to our study because Tan et al. did not measure the effects *within* a patient on- and off-drug, but *between* patients using or not using $Na_V$ blockers chronically, their findings do corroborate the decrease of 5ESN and 5XLSN due to mexiletine that we found. Moreover, although no significant difference in $Lat[15\,Hz]_{Last}$ was found between patients with myotonia congenita and healthy subjects, patients using $Na_V$ blockers did have a significant increase in $Lat[15\,Hz]_{Last}$, in line with our results. $FreqLatMin_{last}$ is significantly decreased in patients using $Na_V$ blockers when compared with patients without these drugs, in line with our findings for mexiletine.

## MVRC as a biomarker in drug development

Our study shows that MVRC end points are suitable to detect drug effects on muscle excitability, even in a small

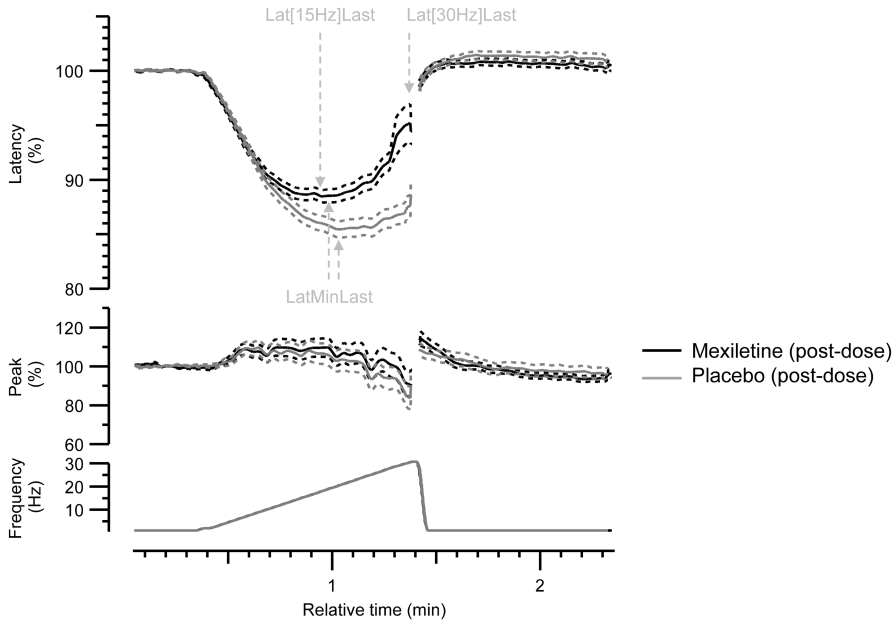


**FIGURE 6** Mean postdose recordings of frequency ramp, for mexiletine (black) and placebo (gray). Dotted lines show the standard error. The upper graph shows the percentual latency (compared to unconditioned latency) by a train of pulses (stimulation rate shown in the lowest graph). The middle graph shows the percentual amplitude change (compared to unconditioned amplitude values). Both graphs show the last-in-train values. Variables with significant effects (mexiletine vs. placebo) are visualized by indicating the name of the variable. Variable visualization is reproduced from ref. [11]. Note this graph is meant to visualize treatment effects, but does not fully reflect the statistical analysis, the statistical model includes baseline as a covariate which is not reflected in the graph.

number of healthy subjects, with a limited number of postdose measurements. The sample size used here is a typical sample size used in phase I studies. Additionally, the MVRC measurement was safe and well-tolerated in this study. The duration of one measurement allows for predose and multiple postdose measurements: the stimulation protocol used in this study takes ~7 min. In addition, the intrasubject variability derived from the model is acceptable, reflected by CV%s below 20% for 17 of 25 variables, which supports the use of MVRC as a biomarker in a cross-over study design. As these test–retest reliability results are based on the data in the placebo treatment, this indicates that the end points were rather stable under placebo (i.e., there was no apparent placebo response). These properties are a prerequisite for a valuable biomarker in early phase clinical trials. Whether effects of compounds developed for various NMDs can be detected using MVRC will have to be confirmed in future studies. However, we propose the use of MVRC as a biomarker for target engagement of drugs developed to influence muscle excitability, such as novel (subtype-specific) $Na_V$ blockers,[19,20] or existing sodium- or potassium channel modulating therapies proposed as new treatments for myotonia.[21–23] This biomarker may therefore be used for proof of target engagement but may also facilitate an informed choice of the dose level in the translation from phase I studies in healthy subjects to phase II and III studies in patient populations. Furthermore, MVRCs may also be used in the translational phase between preclinical and clinical studies because the measurement can also be performed in animal studies.[24,25]

For further development of MVRC as PD biomarker, it would be of interest to explore concentration effect relationships on MVRC. The current study is not set up to reliably evaluate this, because the spread in plasma concentrations is insufficient: we only performed two postdose PD measurements, both at high plasma concentrations.

## LIMITATIONS

Due to potential effects of edema or bleeding around the needle electrodes on consecutive measurements, the insertion location of the needle varied slightly (~0.5 cm) between measurements on the same day. This may influence the conduction distance slightly between measurements performed on the same day. However, intrasubject variability was low, suggesting that this was not a major problem. Moreover, a previous variability study did not report a significant effect of conduction distance on the MVRC end points calculated as percentage latency change.[18]

A potential limitation of MVRC is that it can be challenging to find suitable muscle responses to perform the MVRC measurement. This can lead to technically aberrant measurements that have to be removed from analysis, although this occurred rarely in our dataset (see Section Data handling).

The analyses presented here were not corrected for multiple testing, due to the exploratory nature of the study.

## CONCLUSION

The aim of this study was to evaluate MVRC as a biomarker for PD effects on muscle excitability. We demonstrated significant effects of the use-dependent $Na_V$ channel blocker mexiletine on MVRC in healthy subjects. The results indicate a reduction of muscle excitability by mexiletine, in line with its suggested mechanism of action. Whether MVRC can detect PD effects of other (novel) treatments for NMDs remains to be determined in future work. However, this study encourages the use of MVRC as a tool to demonstrate PD effects of drugs targeting muscle excitability in early phase clinical drug development.

### AUTHOR CONTRIBUTIONS

T.Q.R. and J.A.A.C.H. wrote the manuscript. T.Q.R., M.R.T., G.J.G., and J.A.A.C.H. designed the research. T.Q.R., I.W.K., and G.J.G. performed the research. M.L.dK. analyzed the data.

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### CONFLICT OF INTEREST

M.R.T. has been involved in research sponsored by ArgenX, Alexion Pharmaceuticals, and NMD Pharma, and acts as a scientific advisor to UCB Pharma and ArgenX. He has not benefited personally from these functions; all reimbursements were received by Leiden University Medical Center. He is a member of the European Reference Network for Rare Neuromuscular Diseases. All other authors declared no competing interests for this work.

### DATA AVAILABILITY STATEMENT

The study protocol, and data and scripts that support the findings of this study, are available from the corresponding author upon reasonable request.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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