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Muscle velocity recovery cycles in neurogenic muscles

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HIGHLIGHTS

- Muscle velocity recovery cycles (MVRCs) show in vivo evidence of depolarized muscle membrane in neurogenic muscles in humans.
- MVRC parameters correlate to disease severity and muscle strength but not to disease duration.
- MVRC is a useful technique for revealing disease mechanisms in neuromuscular disorders.

ABSTRACT

Objective: To examine muscle membrane properties in neurogenic muscles using Muscle Velocity Recovery Cycles (MVRCs).
Methods: Forty-seven patients referred to Nerve Conduction Studies (NCS) and Electromyography (EMG) for peroneal nerve entrapment neuropathy were prospectively included. The patients were categorized as peroneal nerve entrapment neuropathy across knee ($n = 22$), L5-radiculapathy ($n = 10$), normal NCS/EMG ($n = 9$) and other disorders ($n = 6$) using NCS/EMG and neuroimaging results. Strength in anterior tibial muscle was measured by Medical Council Scale (MRC) and disease duration was recorded. In addition to conventional NCS/EMG, all subjects were examined with MVRCs in anterior tibial muscle. This provided parameters of muscle relative refractory period (MRRP) and early supernormality (ESN) and late supernormality (LSN). The results were compared with 29 age-matched healthy control subjects.
Results: MRRP was prolonged and ESN and LSN were reduced in neurogenic muscles. MRRP, ESN and LSN correlated to MRC and incidence of spontaneous activity but not to motor unit potential parameters or disease duration.
Conclusions: MVRC changes provide *in vivo* evidence of depolarization in intact human muscle fibres that could underlie reduced muscle excitability and hence weakness in neurogenic muscles.
Significance: MVRCs appear to be a useful technique for revealing disease mechanism in a broad range of neuromuscular diseases.

1. Introduction

Conventional Nerve Conduction Studies (NCS) and Electromyography (EMG) are the methods used for the diagnosis of neuromuscular disorders. While suited for diagnosis these methods provide limited insight into pathophysiological processes and cellular disease mechanisms that may underlie a muscular disease. Understanding muscle fibre membrane properties and cellular alterations in neuromuscular disorders have this far relied on experiments with isolated muscle biopsies from patients. While such experiments with isolated tissue can give detailed insight on disease alterations, it would be of clinical importance to have methodologies to confirm expected disease mechanisms using recordings from intact muscle in patient.

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Recently, Z'Graggen and Bostock developed a simple and fast method for recording muscle velocity recovery cycle (MVRCs). This technique offers *in vivo* assessment of muscle fibre membrane properties by measuring how the velocity of a muscle action potential depends on the time interval after a preceding action potential (Tan et al., 2018).

With this approach it is possible to evaluate muscle dysfunction related to muscle fibre excitability. It can reveal loss of muscle fibre excitability that, in turn, can arise secondarily to prolonged depolarization. Before the invention of this new MVRC method, it was only possible to evaluate muscle fibre depolarization using intracellular electrodes in isolated muscles.

MVRCs are measured by direct stimulation of the muscle and recording from the same bundle of muscle fibres with a sequence of paired pulse electrical stimulations of equal stimulus intensity but varying interstimulus interval (ISI) (Z'Graggen W and Bostock, 2009, Z'Graggen et al., 2011, Bostock et al., 2012). MVRCs can be used to determine muscle relative refractory period (MRRP), the duration when the muscle fibre is refractory immediately after an action potential. It also reveals a supernormal period during which action potentials conduct faster than normal. The supernormal period comprises two phases: early supernormality (ESN) and late supernormality (LSN), which are believed to be related to depolarizing afterpotentials.

MVRCs have been shown to be an indirect albeit effective measure of membrane depolarization in limb ischemia (Z'Graggen and Bostock, 2009) and renal failure (Z'Graggen et al., 2010), and it has been used to provide information about membrane abnormalities in critical illness myopathy (Z'Graggen et al., 2011), Andersen-Tawil syndrome (Tan et al., 2012), myotonia congenita (Tan et al., 2014) and myotonic dystrophy (Tan et al., 2016). Collectively, this demonstrates the wide applicability of MVRC to muscle diseases.

As a result of changes in innervating motor neurons, secondary changes in skeletal muscles develop and such affected muscles are referred to as neurogenic muscle. While evident clinical abnormality is present in neurogenic muscles, the corresponding cellular changes in muscle fibre membrane potentials have been demonstrated in experimental studies of isolated muscle tissue, but have yet to be confirmed in vivo in patients. (Kirsch and Anderson, 1986, Kotsias and Venosa, 1987, Gregorio et al., 1988). MVRC offers a unique possibility to provide further insights into muscle membrane properties in the denervation process in human.

In this study, we examined MVRCs in neurogenic muscles to give a better understanding of the cellular mechanisms that underlie muscle dysfunction in denervation process and demonstrate that the muscle fibres develop pronounced loss of excitability that is compatible with depolarization arising as an important component of the dysfunctions in the neurogenic muscle.

2. Material and methods

2.1. Subjects

The study was conducted prospectively on patients referred with the suspicion of peroneal nerve entrapment neuropathy in the period January 2016 to December 2017 at the Department of Clinical Neurophysiology, Aarhus University Hospital, Denmark. Two patients were excluded because we could not perform any MVRC recording due to severe atrophy of the anterior tibial muscle. The analyses were done in 47 patients (mean age: 55.00 ± 18.00, 24 males and 23 females). The results were compared with 29 healthy controls (mean age: 55.7 ± 14.9, 14 males and 15 females).

The study was approved by the Regional Scientific Ethical Committee and the Danish Data Protection Agency. Informed written consent was obtained from all patients and controls according to the Declarations of Helsinki.

2.2. Clinical examination

Detailed neurological examination including evaluation of muscle strength, deep tendon reflexes and sensory examination for touch, pinprick and vibration sense were done on all subjects. The strength in the anterior tibial muscle was evaluated using Medical Research Council (MRC) scale differing between 0 (paralysis) and 5 (normal strength).

2.3. Nerve conduction studies (NCS)

The EMG-equipment Keypoint version 2.11 (Dantec, Skovlunde, Denmark) was used for NCS measurements. Routine motor and sensory NCS were performed in all patients on the peroneal nerve as a part of diagnostic evaluation. Motor NCS was performed by delivering supramaximal stimulation at the ankle, below capitulum fibulae and in fossa poplitea while recording from the extensor digitorum brevis muscle. Similarly, motor NCS was done by delivering supramaximal stimulation below capitulum fibulae and in fossa poplitea while recording from the anterior tibial and peroneus longus muscles using concentric needles. Sensory NCS was performed either by near nerve recordings or surface electrode techniques. With near-nerve needle technique, the superficial peroneal nerve was stimulated by supramaximal stimulation at retinaculum superior and sensory nerve action potentials (SNAPs) were recorded orthodromically below capitulum fibulae and in fossa poplitea using needle electrodes placed close to the nerve (Tankisi et al., 2014). With surface electrode technique, the superficial peroneal nerve was stimulated at lateral crus and SNAPs were recorded at retinaculum superior. The evaluated parameters for motor NCS were distal motor latency, conduction velocity (CV), compound muscle action potential (CMAP) amplitude and minimum F-wave latency. For sensory NCS, CV and SNAP amplitude were evaluated. NCS results were compared with laboratory normal material. The diagnosis of peroneal nerve entrapment neuropathy has been given by a significant drop in CV or in CMAP amplitude or both across the knee indicating focal demyelination or conduction block (Marciniak, 2013). Entrapment neuropathy of the peroneal nerve was defined if one or more of the following criteria (unpublished) developed by Danish Consensus Group were full-filled: (1) motor conduction block across the knee, (2) decreased motor or sensory CVs across the knee while CVs were normal at lower leg, (3) decreased motor or sensory CV across the knee and at the lower leg if there was more than 10 m/sec decrease across the knee compared with lower leg.

CVs less than mean −2SD were considered as abnormal according to laboratory normal material. Conduction block was defined as more than 50% decrease in amplitude recorded in extensor digitorum brevis muscle between the two stimulation sites, capitulum fibulae and fossa poplitea (Tankisi et al., 2005).

In addition to NCS of the peroneal nerve, the tibial and sural nerves were examined in some patients as part of the diagnostic evaluation but these data are not presented here.

In healthy controls, only peroneal motor NCS with surface electrodes were done recording from extensor digitorum brevis muscle.

2.4. Electromyography (EMG)

In all patients and healthy subjects, EMG in the anterior tibial muscle was done using a concentric 35-mm Dantec needle electrode and the EMG-equipment Keypoint version 2.11 (Dantec, Skovlunde, Denmark). Standard filter settings at the department

(20 Hz–10 kHz), gain (100 μV/division) and sweep speed (10 ms/division) were used.

In some patients, EMG of other muscles was done for diagnostic purposes but the data are not presented here.

2.4.1. Evaluation of spontaneous activity

The presence of fibrillation potentials (fibs), positive sharp waves (PSWs) and fasciculations was assessed in 10 separate sites for 60–90 seconds at each site. All types of spontaneous acitivity, i.e. fibs, PSWs, fasciculations at each site were noted and the frequency of spontaneous activity out of 10 sites was provided. Fibs or PSWs in more than two sites were regarded as abnormal (Tankisi et al., 2007).

2.4.2. Quantitative motor unit potential (MUP) analysis

Quantitative MUP analysis (Buchthal et al., 1954, Fuglsang-Frederiksen et al., 2016) was done by sampling of at least 20 potentials sampled during weak effort, corresponding to about 4% of maximal voluntary contraction (Ronager et al., 1989). Mean duration and amplitude were evaluated both in patients and healthy controls.

2.5. Muscle velocity recovery cycles (MVRCs)

MVRCs were recorded in the anterior tibial muscle in all patients and healthy subjects using the nerve and muscle excitability set-up as previously described (Z'Graggen and Bostock, 2009). Briefly, the stimulating cathode, an insulated monopolar needle electrode (26G, TECA) was inserted perpendicular to the middle or last third of the muscle. A non-depolarizing surface electrode serving as the anode was placed one centimeter distal to the stimulating cathode. Muscle activity was recorded 20 millimeters proximal to the stimulating needle electrode by a concentric 25-mm Dantec needle electrode. The ground electrode was placed distal to the stimulating needle electrode. Skin temperature was kept between 32 and 35 degrees using a heating lamp.

Stimulations were delivered by an isolated constant current-stimulator (DS5; Digitimer Lt, Welwyn Garden City, Hertfordshire, UK). The signal was amplified (gain 300–1000, bandwidth 0.2 Hz to 3 kHz) and digitized (NIDAQ-6062E, National Instruments Europe Corp.) using a sampling rate of 20 kHz. The electrodes were adjusted to obtain a stable negative peak response with a stimulus intensity of 3–10 mA of 0.05 ms rectangular current pulses. Stimulation and recordings were controlled by Qtrac software (written by Hugh Bostock, Institute of Neurology, London, UK), using the M3RC3 recording protocol.

By the M3RC3 protocol, MVRCs were recorded with 1, 2 and 5 conditioning stimuli 10 ms apart. Conditioning stimuli were separated from the test pulses by inter-stimulusintervals (ISI) that varied from 1000 to 1.4 ms in 34 steps. Test stimuli were delivered every 2 seconds.

2.6. Statistical analysis

To evaluate the relation between the muscle strength, EMG results and MVRC parameters, Spearman Rank correlation analysis was performed. Parametric t-test or non-parametric Mann-Whitney test was used depending on whether the data was normally distributed or not to compare patients and healthy controls. Proportions were compared using chi-square statistics. QtracP was used for the statistical analyses and for generating the figures. Bonferroni's correction was performed to minimize type I errors with the formula of significance level = 0.05/number of tests performed. We had 7 MVRC parameters tested. Therefore, a p-value of <0.007 was required after Bonferroni's correction.

3. Results

3.1. MVRC in different patient groups

Patient demographics and clinical characteristics are reported in Table 1. There was no statistically significant difference in age (t test, $p = 0.85$) and sex (chi-square test, $p = 0.77$) between patients and healthy controls.

By use of NCS, EMG and imaging results, patients were categorized in 4 groups: (1) Patients with a localised peroneal nerve entrapment neuropathy across the knee ($n = 22$), (2) Patients with magnetic resonance imaging and electrophysiologically confirmed L5 radiculopathy ($n = 10$), (3) Patients with normal NCS and EMG ($n = 9$), and (4) Patients with other disorders ($n = 6$) (non-localized peroneal nerve mononeuropathy due to vasculitis ($n = 2$) or idiopathic aetiology ($n = 1$), polyneuropathy ($n = 2$) and amyotrophic lateral sclerosis ($n = 1$)).

Table 1
Patient demographics and clinical characteristics.

			4 patient subgroups *(n = 47)*				Patients with profuse denervation activity *(n = 14)*
	Healthy controls *(n = 29)*	All patients *(n = 47)*	Peroneal nerve entrapment neuropathy *(n = 22)*	L5 root affection *(n = 10)*	Patient with normal NCS/EMG *(n = 9)*	Patients with other disease *(n = 6)*	
Age (Years)	55.7 ± 14.9	55.0 ± 18.0	54.8 ± 18.9	52.8 ± 19.1	49.0 ± 19.0	68.5 ± 5.1	58.9 ± 16.3
Gender (M/F)	14/15	24/23	12/10	7/3	3/6	2/4	9/5
Disease duration (Months)	-	7.7 ± 9.9	3.6 ± 3.5	10.6 ± 9.4	16.9 ± 16.4	3.6 ± 2.1	3.4 ± 2.7
MRC score	–	3.8 ± 1.1	3.4 ± 1.2	3.7 ± 1.0	4.9 ± 0.3	3.8 ± 0.3	3.0 ± 1.1
Etiology	–	–	Weight loss (11) Sitting position (13)[*] Trauma (1) Idiopatic (3)	L5 root (10)	Pain (9)[§] Psycogenic decreased force (1)	Non-localised peroneus neuropathy (3)[Ω] PNP (2) ALS (1)	Peroneus neuropathy (9)[#] L5 root (5)

[#] 8 patients had localised peroneal nerve entrapment neuropathy and 1 had non-localised (due to vasculitis) peroneus neuropathy.

[*] 10 patients had the habit of crossing the legs regularly (6 of these patients had weight loss too), 1 patient had a 20 h bus trip, 1 sat long in lotus position, 1 worked long on knees.

[§] 5 patients had musculoskeletal pain in ankle/foot and 3 patients had unspecific diffuse pain/sensory disturbances in leg. 1 patient had both pain and psycogenic decreased force.

[Ω] 3 patients had non-localised peroneus neuropathy (2 due to vasculitis and 1 idiopathic). PNP = Polyneuropathy, ALS = Amyotrophic lateral sclerosis. Values are listed as means ± standard deviation (SD) of the mean.

Table 2
Comparison of MVRC and MUP parameters between different patient groups and healthy controls.

	(Mean ± SD)					*P* values for t-test			
	All patients (*n* = 47)	Peroneal neuropathy (*n* = 22)	Radiculopathy (*n* = 10)	Normal NCS/ EMG (*n* = 9)	Healthy controls (HC) (*n* = 29)	All patients vs HC	Peroneal neuropathy vs HC	Radiculopathy vs HC	Normal NCS/ EMG vs HC
MRRP (ms)	4.8 ± 1.8	5.3 ± 1.9	4.5 ± 1.5	3.6 ± 0.5	3.5 ± 0.4	**$p = 5.3^{-4}$**	**$p = 2.7^{-5}$**	**$p = 0.005$**	$p = 0.357$
ESN (%)	9.7 ± 3.3	9.0 ± 3.9	9.1 ± 2.1	10.7 ± 2.7	11.3 ± 2.0	$p = 0.019$	$p = 0.009$	$p = 0.012$	$p = 0.474$
ESN (ms)	9.5 ± 2.4	10.3 ± 2.1	8.9 ± 2.4	7.3 ± 1.0	7.8 ± 2.1	**$p = 0.003$**	**$p = 1.8^{-4}$**	$p = 0.239$	$p = 0.570$
5ESN (%)	10.1 ± 4.3	8.6 ± 4.8	10.2 ± 3.0	13.0 ± 3.4	13.8 ± 2.5	**$p = 1.0^{-4}$**	**$p = 1.7^{-5}$**	**$p = 0.002$**	$p = 0.436$
LSN (%)	3.6 ± 1.5	3.3 ± 1.5	3.3 ± 1.4	4.5 ± 1.1	4.1 ± 1.8	$p = 0.160$	$p = 0.06$	$p = 0.172$	$p = 0.621$
XLSN (%)	2.0 ± 1.0	1.6 ± 0.9	1.8 ± 0.8	3.0 ± 1.1	2.8 ± 0.7	**$p = 2.8^{-4}$**	**$p = 1.2^{-5}$**	**$p = 0.001$**	$p = 0.843$
5XLSN (%)	5.3 ± 2.5	4.7 ± 2.3	4.9 ± 2.5	7.1 ± 2.2	8.0 ± 1.5	**$p = 4.0^{-6}$**	**$p = 5.6^{-7}$**	**$p = 9.3^{-5}$**	$p = 0.669$
MUP dur (ms)	16.4 ± 3.0	17.1 ± 2.9	17.1 ± 2.3	13.3 ± 1.2	13.3 ± 1.2	**$p = 4.2^{-6}$**	**$p = 4.7^{-7}$**	**$p = 3.7^{-7}$**	$p = 0.520$
MUP amp (mV)	0.6 ± 0.3	0.6 ± 0.3	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.09	**$p = 4.7^{-4}$**	**$p = 1.3^{-4}$**	**$p = 2.4^{-6}$**	$p = 0.825$

MVRC = Muscle Velocity Recovery Cycle; MRRP = muscle relative refractory period; ESN = early supernormality (up to 15 ms); ESN (ms) = inter-stimulus interval for maximum ESN; LSN = late supernormality (50–150 ms); 5XLSN etc = extra supernormality after 5 conditioning stimuli compared with 1 conditioning stimulus; MUP dur (ms) = motor unit potential duration; MUP amp (mV) = motor unit potential amplitude. *P* values of <0.007 are in bold type after Bonferroni's corrections. Values listed are means ± standard deviation (SD) of the mean. Group analyses for patients with other disorders (*n* = 6) were not done because of low number of patients in this group.

When looking at all patients as a group, MVRC parameters showed depolarization with prolonged MRRP and decreased ESN and LSN compared with healthy controls (Table 2, Fig. 1A–C).

In patients with localised peroneal nerve entrapment neuropathy and L5 radiculopathy, the same pattern as for the whole patient group was seen with significantly prolonged MRRP and decrease in ESN and LSN (Table 2). These differences compared with healthy controls were however not found in patients with normal NCS and EMG (Table 2, Fig. 1D–F). MUP duration and MUP amplitude were also significantly different in peroneal nerve entrapment neuropathy and L5 radiculopathy (Table 2). None of the MVRC parameters differed between the patients with localised peroneal nerve entrapment neuropathy and radiculopathy. Group analyses were not done for the group with other disorders due to the small number of patients in this group.

Patients with peroneal nerve entrapment neuropathy were divided into two groups as: (1A) patients with conduction block (*n* = 9 patients) and (1B) patients without conduction block (*n* = 13 patients). Both groups showed prolonged MRRP and decrease in ESN and LSN compared with healthy controls whereas there was no significant difference in any of the MVRC parameters between patients with and without conduction block (Table 3). Large variations between subjects in MVRC curves were seen in both subgroups (Fig. 2).

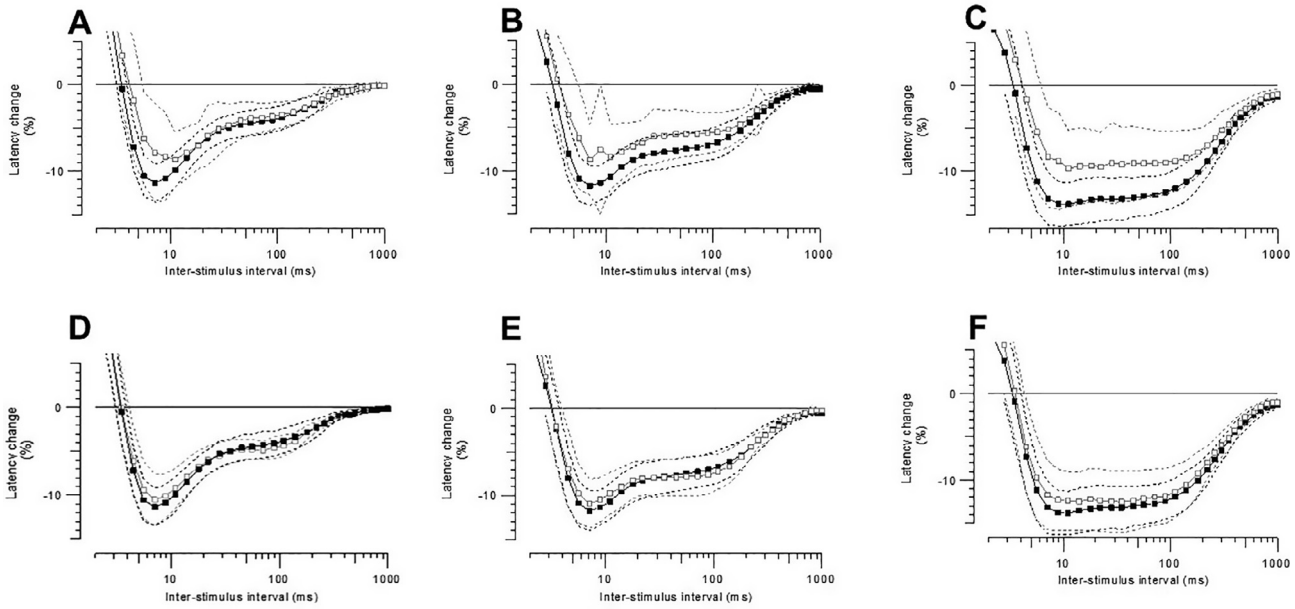

Fig. 1. Muscle velocity recovery cycles (MVRCs) with 1, 2 and 5 conditioning stimuli depicted as mean ± SD. (A) MVRCs in all patients referred with the suspicion of peroneal nerve neuropathy (*n* = 47, open grey squares) compared with healthy controls (*n* = 29, filled black squares). Percentage change in latency is plotted against ISIs from 2 to 1000 ms (logarithmic scale). (B, C) As A, but with 2 and 5 conditioning stimuli. (D–F) Corresponding plots comparing patients with normal nerve conduction studies and electromyography (*n* = 9, open grey squares) compared with healthy controls (*n* = 29, filled black squares).

Table 3
MVRC parameters in peroneal nerve entrapment neuropathy with and without conduction block (CB) compared to healthy controls.

		(Mean ±SD)		*P* values for t-test		
	Peroneal neuropathy with CB (n = 9)	Peroneal neuropathy without CB (n = 13)	Healthy controls (HC)	Peroneal neuropathy with CB vs HC	Peroneal neuropathy without CB vs HC	Peroneal neuropathy with CB vs without CB
MRRP (ms)	5.5 ± 1.9	5.1 ± 2.0	3.5 ± 0.4	**$p = 1.5^{-5}$**	**$p = 1.7^{-4}$**	p = 0.691
ESN (%)	7.9 ± 3.8	9.8 ± 3.9	11.3 ± 2.0	**$p = 0.001$**	p = 0.117	p = 0.272
ESN (ms)	10.7 ± 2.1	9.9 ± 2.1	7.8 ± 2.1	**$p = 8.3^{-4}$**	**$p = 0.005$**	p = 0.400
5ESN (%)	7.2 ± 4.7	9.7 ± 4.7	13.8 ± 2.5	**$p = 6.3^{-6}$**	**$p = 8.9^{-4}$**	p = 0.254
LSN (%)	3.1 ± 0.9	3.3 ± 1.8	4.1 ± 1.8	p = 0.108	p = 0.178	p = 0.758
XLSN (%)	1.8 ± 0.7	1.5 ± 1.0	2.8 ± 0.7	**$p = 0.001$**	**$p = 5.2^{-5}$**	p = 0.445
5XLSN (%)	4.9 ± 2.1	4.6 ± 2.5	8.0 ± 1.5	**$p = 3.6^{-5}$**	**$p = 6.1^{-6}$**	p = 0.736

MVRC = Muscle Velocity Recovery Cycle; MRRP = muscle relative refractory period; ESN = early supernormality (up to 15 ms); ESN (ms) = inter-stimulus interval for maximum ESN; LSN = late supernormality (50–150 ms); 5XLSN etc = extra supernormality after 5 conditioning stimuli compared with 1 conditioning stimulus. *P* values of <0.007 are in bold type after Bonferroni's corrections. Values listed are means ± standard deviation (SD) of the mean.

3.2. Correlation between disease duration and MVRC parameters

Disease duration was variable between patients in the whole patient group (mean ± SD: 7.7 ± 9.9 months, range: 1 week-5 years). A similar variation was observed in subgroups of patients with radiculopathy (mean ± SD: 10.6 ± 9.4 months, range: 5 weeks-24 months) and localized peroneal nerve lesion (mean ± SD: 3.6 ± 3.5 months, range: 1 week-10 months).

Most patients were examined in subacute or chronic phases of their disease. Out of 47 patients, there were only 8 patients examined after ≤4 weeks of disease duration. There was no difference in any of the MVRC parameters between patients with ≤4 weeks disease duration or >4 weeks ($p > 0.05$). There was no correlation between disease duration and MVRC parameters neither in the whole patient group nor in subgroups of radiculopathy or localized peroneal nerve lesion ($p > 0.05$). Similarly, no correlation was seen between MVRC parameters and disease duration in the peroneal neuropathy subgroups with and without conduction block ($p > 0.05$). In Fig. 3, the variability of MVRC curves independent of disease duration is illustrated in four peroneal nerve entrapment neuropathy patients with conduction block compared with the mean for healthy controls.

Like peroneal neuropathy, MVRC curves were altered in patients with radiculopathy (Fig. 4) without any significant correlation to disease duration.

The only electrophysiological parameter that correlated with disease duration was the incidence of fibs/PSWs (Rho = −0.41, $p = 0.004$) for the whole patient group, whereas no correlation was found for the subgroups of patients ($p > 0.05$).

3.3. Correlation between muscle strength, EMG results and MVRC parameters

The correlations between the muscle strength, EMG results and MVRC parameters are summarized in Table 4. The strength in the anterior tibial muscle according to MRC scale correlated significantly to MRRP, ESN and LSN. The incidence of fibs/PSWs correlated significantly to MRRP, ESN and LSN. MUP duration did not correlate to any of the MVRC parameters while MUP amplitude

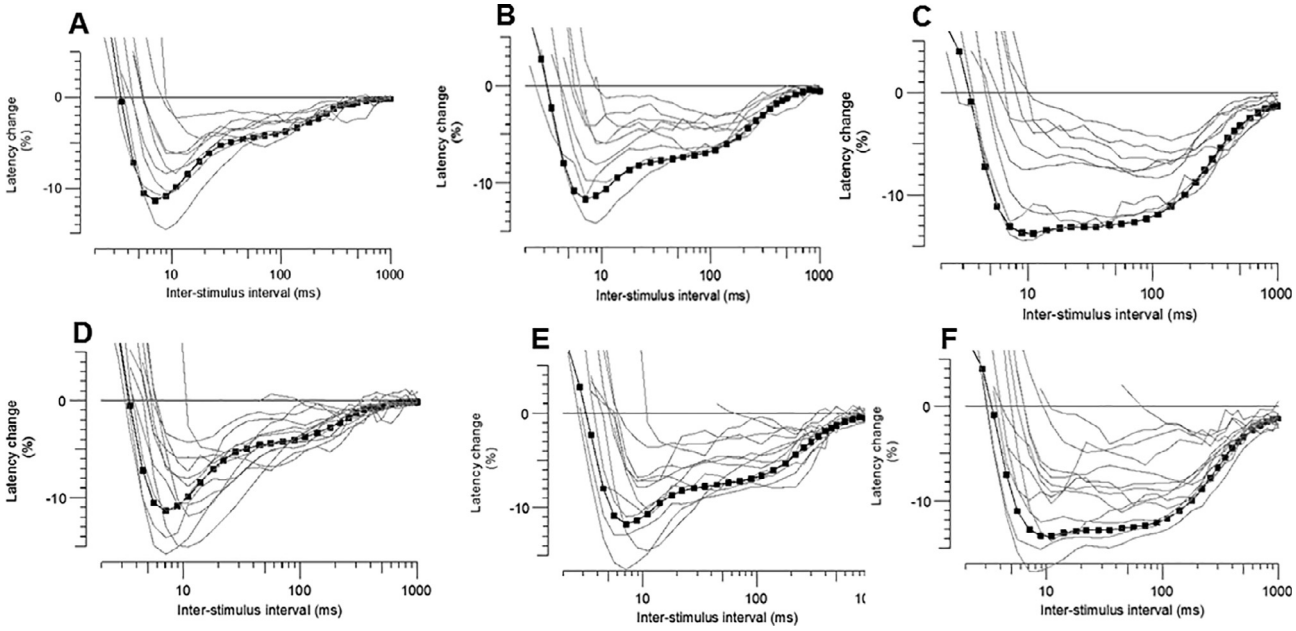

Fig. 2. Muscle velocity recovery cycles (MVRCs) with 1, 2 and 5 conditioning stimuli. (A) MVRCs in peroneal nerve entrapment neuropathy with conduction block (CB) (n = 9, grey lines) compared with healthy controls (n = 29, filled black squares). Percentage change in latency is plotted against ISIs from 2 to 1000 ms (logarithmic scale). (B, C) As A, but with 2 and 5 conditioning stimuli. (D–F) Corresponding plots comparing patients with peroneal nerve entrapment neuropathy without CB (n = 13, grey lines) with healthy controls (n = 29, filled black squares).

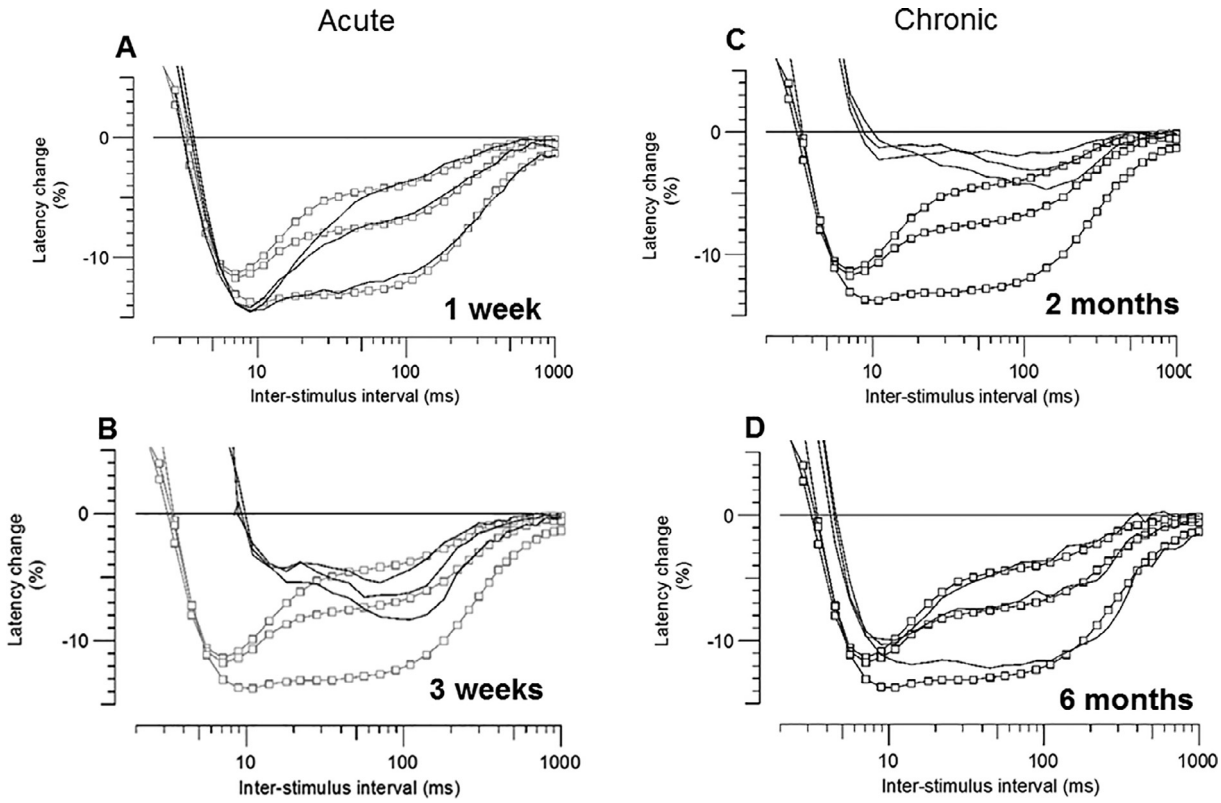

Fig. 3. Muscle velocity recovery cycles (MVRCs) from 4 different patients with 1, 2 and 5 conditioning stimuli. MVRCs in patients with peroneal nerve entrapment neuropathy with conduction block (grey lines) examined in acute (A and B) and chronic (C and D) phases compared with healthy controls (n = 29, filled black squares). Percentage change in latency is plotted against ISIs from 2 to 1000 ms (logarithmic scale). Disease duration is indicated on the right bottom of each figure.

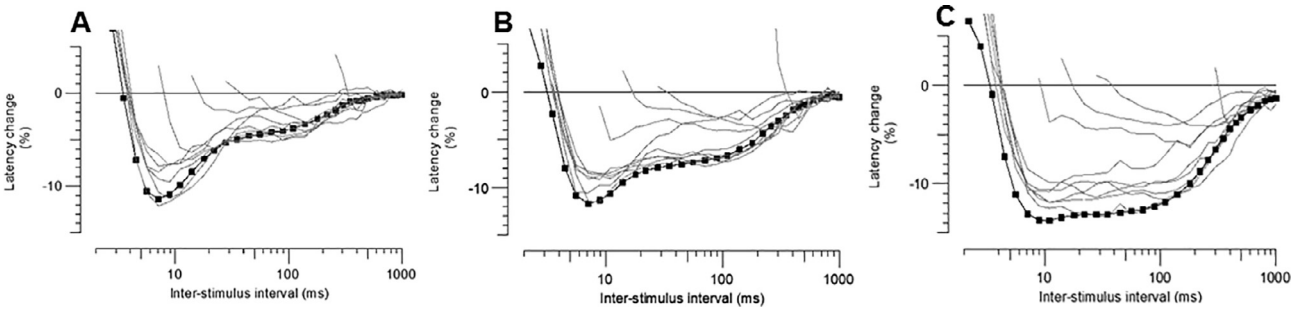

Fig. 4. Muscle velocity recovery cycles (MVRCs) with 1, 2 and 5 conditioning stimuli. (A) MVRCs in patients with L5 radiculopathy (n = 10, grey lines) compared with healthy controls (n = 29, filled black squares). Percentage change in latency is plotted against ISIs from 2 to 1000 ms (logarithmic scale). (B, C) As A, but with 2 and 5 conditioning stimuli.

correlated only to LSN (Table 4). There was no correlation between the incidence of fasciculations and MVRCs for any of the parameters (p > 0.007).

3.4. MVRC in chronic neurogenic muscles without spontaneous activity

In 11 patients, there were no fibs/PSWs but there were signs of chronic neurogenic changes with increased MUP duration or amplitude. These patients' MVRCs showed also significant prolonged MRRP and decrease in ESN and LSN compared with healthy subjects similar to the whole group of patients (Table 5).

3.5. MVRC in muscles with profuse denervation activity

In 14 patients, there were fibs/PSWs in all sites showing profuse denervation activity. These patients' MVRC showed the most prominent prolongation in MRRP and decrease in ESN and LSN (Table 5).

4. Discussion

MVRCs provide information about changes in muscle fiber membrane properties that depend on the resting membrane potential of muscle fibers. The methodology has in this paper been used to obtain information on the effect of the denervation process on the muscle fibers. The method has previously been validated by showing to be of value in various myopathies and muscle channelopathies (Z'Graggen et al., 2010, Z'Graggen et al., 2011, Tan et al., 2012, Tan et al., 2016). In this study, we applied MVRCs for the first time in neurogenic muscles. MVRCs in different types of neurogenic disorders differed from healthy subjects while MVRCs were normal in patients with normal NCS and EMG. MVRC parameters correlated to EMG parameters particularly to the incidence of fibs/PSWs, however there were also MVRC changes in patients with neurogenic muscles without fibs/PSWs.

Table 4
Correlations between the muscle strength, EMG results and MVRC parameters.

	Patients							
	MRC		Fibs/PSWs		MUP duration		MUP amplitude	
	Rho	*p*-value	Rho	*p*-value	Rho	*p*-value	Rho	*p*-value
MRRP (ms)	−0.49	**0.001**	0.51	**0.001**	0.40	0.011	0.34	0.012
ESN (%)	0.25	0.112	−0.16	0.301	−0.10	0.543	−0.09	0.603
ESN@ (ms)	−0.44	**0.004**	0.53	**3.5^{-4}**	0.39	0.013	0.33	0.038
5ESN (%)	0.47	**0.002**	−0.46	**0.002**	−0.20	0.215	−0.20	0.234
LSN (%)	0.44	**0.002**	−0.49	**0.001**	−0.33	0.029	−0.44	**0.004**
XLSN (%)	0.49	**0.001**	−0.56	**8.1^{-5}**	−0.32	0.037	−0.28	0.071
5XLSN (%)	0.45	**0.002**	−0.51	**2.1^{-4}**	−0.28	0.064	−0.23	0.129

EMG = Electromyography; MVRC = Muscle Velocity Recovery Cycle; MRC = Medical Research Council; Fibs/PSWs = Fibrillations or positive sharp waves; MUP = Motor Unit Potential; MRRP = muscle relative refractory period; ESN = early supernormality (up to 15 ms); ESN (ms) = inter-stimulus interval for maximum ESN; LSN = late supernormality (50–150 ms); 5XLSN etc = extra supernormality after 5 conditioning stimuli compared with 1 conditioning stimulus. *P* values of <0.007 are in bold type after Bonferroni's corrections.

Table 5
Comparison of MVRC and quantitative motor unit potential (MUP) analysis parameters between healthy controls and patients with chronic neurogenic muscles (muscles without spontaneous activity) and patients with profuse denervation activity.

	(Mean ± SD)			*p*-value for t-test	
	Chronic neurogenic muscles ($n = 11$)	Healthy controls (HC) ($n = 29$)	Muscles with profuse denervation activity ($n = 14$)	Chronic neurogenic muscles vs HC	Muscles with profuse denervation activity vs HC
MRRP (ms)	4.3 ± 0.6	3.5 ± 0.4	7.6 ± 3.1	**$p = 9.9^{-5}$**	**$p = 6.8^{-8}$**
ESN (%)	9.5 ± 2.7	11.3 ± 2.1	7.6 ± 2.3	$p = 0.027$	**$p = 5.5^{-5}$**
ESN (ms)	9.2 ± 1.4	7.8 ± 1.3	12.7 ± 1.6	$p = 0.049$	**$p = 1.6^{-8}$**
5ESN (%)	10.8 ± 2.5	13.7 ± 2.5	5.4 ± 2.5	**$p = 0.002$**	**$p = 9.3^{-10}$**
LSN (%)	4.1 ± 1.0	4.1 ± 1.4	2.7 ± 1.7	$p = 0.879$	$p = 0.017$
XLSN (%)	2.2 ± 0.6	2.9 ± 0.7	1.0 ± 0.6	$p = 0.019$	**$p = 1.8^{-10}$**
5XLSN (%)	6.2 ± 1.5	8.0 ± 1.4	2.8 ± 1.6	**$p = 0.002$**	**$p = 2.2^{-11}$**
MUP duration (ms)	17.05 ± 2.3	13.3 ± 1.2	18.7 ± 2.9	**$p = 3.3^{-7}$**	**$p = 1.5^{-8}$**
MUP amplitude (mV)	0.7 ± 0.4	0.4 ± 0.1	0.6 ± 0.2	**$p = 5.4^{-4}$**	**$P = 3.9^{-7}$**

MVRC = Muscle Velocity Recovery Cycle; MRRP = muscle relative refractory period; ESN = early supernormality (up to 15 ms); ESN (ms) = inter-stimulus interval for maximum ESN; LSN = late supernormality (50–150 ms); 5XLSN etc = extra supernormality after 5 conditioning stimuli compared with 1 conditioning stimulus. *P* values of <0.007 are in bold type after Bonferroni's corrections. Values listed are means ± standard deviations (SD) of the mean.

4.1. MVRC findings are compatible with depolarization of the resting membrane potential

In this study MVRCs showed changes in the neurogenic muscle that are compatible with depolarization of the resting membrane potential of the muscle fibers. Muscle membrane depolarization in denervated muscles has been previously shown in experimental studies using isolated muscle (Kirsch and Anderson, 1986, Kotsias and Venosa, 1987, Gregorio et al., 1988) but confirmation of this has not previously been obtained from intact muscle in patients. MVRC recordings in the present study provide indirect evidence that confirm depolarization in the neurogenic muscle. Hence, voltage gated Na^+ channels are responsible for excitation and propagation of muscle fiber action potentials and these channels are decisive for the MRRP in muscle fibers. The channels are voltage dependent and prolonged depolarization leads to an increased probability of the channels entering a slow-inactivated state that prevents the opening of the channels and contributes to formation of the action potential in muscle fibers (Ruff et al., 1988). Such reduction in the function of voltage gated Na^+ channels increases the relative refractory period. The marked and consistent increase in MRRP in the MVRC recordings across patient groups compared with healthy controls thus agrees with depolarization being an important aspect of the disease processes in neurogenic muscle.

4.2. Correlation between disease duration and MVRC parameters

Disease duration in our patient group varied between 1 week and 60 months. MVRC parameters also varied markedly from normal to severely affected. However, we did not find any correlation between disease duration and MVRC parameters. Further studies with larger groups of patients examined within the early days of disease onset and followed by repeated recordings are needed. We believe, the variability in MVRC parameters is mainly related to the severity of the nerve lesion.

4.3. MVRC parameters correlate to denervation activity in EMG

Fibs/PSWs are related to the changes in electrical properties of the membrane of denervated muscle fibres and represent foci of depolarization, originating from single muscle fibers with unstable resting membrane potentials (Kirsch and Anderson, 1986, Dumitru and Santa Maria, 2007). These occur just adjacent to a peri-electrode-induced region of membrane crush, which then propagates away from the electrode. (Dumitru and Santa Maria, 2007).

In this study, we quantified the incidence of spontaneous activity and we did quantitative MUP analysis. Fibs/PSWs correlated to MRRP and early and late supernormality in MVRCs. However, correlations were weak which may be explained by the fact that fibs/PSWs were recorded in different sites in the muscle while MVRCs were recorded in one site, which may not be representative for the whole muscle. Additionally, muscle membrane potential may be depolarized even in the absence of spontaneous activity as we showed in muscles with chronic neurogenic changes without fibs and PSWs. The only correlation between MVRC and MUP parameters was between MUP amplitude and LSN. As aforementioned, this may be because MVRC and MUP analyses were not done at the same site or because these show different pathophysiological

processes in the muscle. Another reason for this weak correlation may be that in case of very severe axonal loss resulting in denervation but little reinnervation as seen in some patients, one would not expect large MUPs with long duration and high amplitude.

4.4. Correlation between MVRC parameters and muscle weakness

Muscle weakness was clearly correlated to MVRC parameters. This correlation may reflect that muscle fibers are losing excitability secondary to depolarization and sodium channel slow-inactivation, and this could affect muscle strength in several ways. First, action potential formation is an essential element in the normal activation process of skeletal muscle fibers and failure in the excitation or propagation of action potentials result in functional paralysis and weakness. Second, excitation or propagation of action potentials results in functional paralysis and weakness. Second, even if the fibers can fire action potential, MRRP was a consistent MVRC finding in neurogenic muscle. This would prevent the patients from generating action potentials in the muscle fibers at a high frequency. It is well described that normal muscle activation during voluntary movements often proceed with a motor programming that contain an initial doublet or triplet of high frequency action potential firing, often above 250 Hz (Cheng et al., 2013). These initial high frequency action potentials are important for determining the speed of force production and failing to do so will result in muscle weakness (Cometti et al., 2016). The prolonged MRRP could thus prevent normal muscle activation with initial high frequency action potentials to occur. Such altered motor programming may clinically present as weakness. Moreover, spinal cord circuits have an important role in determining recruitment rate.

5. Limitations

Although we had a large cohort, the number of patients in the subgroups was limited. Particularly the groups of patients with conduction block and L5 radiculopathy were very small. Additionally, our examination methods did not enable us to measure whether the conduction block was caused by only demyelination or alongside with secondary axonal loss. We used in this study MRC score for evaluation of the strength and using a dynamometer could be a more sensitive measure. Another limitation of our study and the MVRC method was performing the recording in only one site and examining only few muscle fibers which does not necessarily represent the whole muscle. Therefore, it would be ideal to test if the examined muscle fibers are denervated, affected by a conduction block of the innervating nerve fibers or normally innervated. Further studies examining denervation activity at the site MVRCs are recorded and stimulation of the nerve while recording from the same site are necessary to address these questions. The limitation of recording from a few muscle fibers in MVRCs is particularly important in disorders where the pathology is not diffuse. Since MVRC examination is not an unpleasant test and takes only 10 minutes, examination of more sites in a muscle may easily be carried out and future studies with recordings from multiple sites should be conducted.

6. Conclusions

We showed in this study that MVRCs suggest depolarised resting potential in denervated human muscles and this depolarization may lead to loss of muscle excitability and reduced muscle strength. There were no differences between subgroups. However, further studies examining muscle membrane properties in different neuromuscular disorders in larger study groups and particularly in acute stage of the disease are needed to explore the potential of MVRCs in clinical settings.

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Declaration of Competing Interest

Authors have no potential conflicts of interest. All authors have approved the final article.

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