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The recovery cycle of excitability assessed by a conventional electrodiagnostic machine: A study in healthy volunteers and in Charcot-Marie-Tooth 1A patients

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HIGHLIGHTS

- The study of the axonal excitability recovery cycle with a conventional machine is feasible and reliable.
- In patients with Charcot-Marie-Tooth (CMT) disease type 1A, the refractory periods and the superexcitable period are reduced.
- The most relevant parameter to discriminate CMT1A from the control group is the area under the curve during superexcitability.

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ABSTRACT

Objective: To validate the 'paired pulses' technique with a conventional electrodiagnostic machine (CEM) for studying the axonal excitability recovery cycle (ERC).
Methods: Paired pulses, with a variable inter-stimulus interval, were delivered at the wrist along the median nerve. The CEM repeatability was verified in a group of 15 healthy volunteers (test/retest analysis). ERC was then applied in 40 healthy volunteers and 10 patients with Charcot-Marie-Tooth type 1A (CMT1A), using both the threshold tracking (TT) reference method and CEM (basal condition, during and after ischemia).
Results: CEM parameters evaluating absolute refractory and supernormal periods were reproducible (interclass correlation coefficient > 0.75). CEM results were consistent with TT method and literature data. In CMT1A, refractory and superexcitable periods were significantly reduced. According to receiving operator characteristic analysis, the CEM supernormal period area was the most relevant parameter for discriminating CMT1A from healthy volunteers (area under the curve = 0.98).
Conclusions: CEM was a valid procedure for studying ERC. CMT1A patients exhibited ERC alterations due to modifications in passive membrane properties and of nodal ion channel distribution resulting from demyelination.
Significance: Studying ERC with CEM could be performed in routine practice in patients with peripheral neuropathies to provide information on motor axonal excitability.

1. Introduction

To date, numerous studies have been conducted to investigate the excitability of peripheral motor or sensory axons. These studies have led to a better understanding of axonal membrane properties at the site of stimulation (ion channel function and axonal membrane potential) (Kiernan et al., 2020).

More than fifty years ago, studies conducted on single nerve fibers documented the excitability recovery cycle (ERC), in other words changes in axonal excitability following the propagation of an action potential (Bergmans, 1970). The sequence of these changes includes four periods. First, the axon is unexcitable, which is the absolute refractory period (ARP). Then, excitability progressively recovers but the axon is hypoexcitable, known as the relative refractory period (RRP). These refractory periods are followed by a supernormal period where axonal excitability is increased and finally by a late subnormal period where axonal excitability is

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decreased. These four periods can be evaluated using paired pulse with varying inter-stimuli interval (Boërio et al., 2004, 2005; Gilliatt and Willison, 1963; Kopec et al., 1978). The exit from the refractory period is mainly related to the recovery of Na^+ channels inactivation (Hodgkin and Huxley, 1952). The supernormal period arises from a "back-flow" of current from the internodal membrane which depolarizes the node (Barrett and Barrett, 1982). The late subnormal period arises from hyperpolarization of the membrane due to the opening of slow K^+ channels.

Modifications of the ERC in patients with demyelinating peripheral neuropathies have been studied using threshold tracking (TT) techniques. In the classic demyelinating form Guillain-Barre syndrome, the ERC is not altered, but in the axonal form, the refractory period is prolonged, suggesting that the function of nodal sodium channels is directly affected by anti-ganglioside antibodies or secondarily by the inflammatory reaction associated with complement activation (Kuwabara et al., 2002). In Charcot-Marie-Tooth type 1A (CMT1A) disease and chronic inflammatory demyelinating polyneuropathies, there is a reduction in refractoriness and superexcitability, presumably due to change in passive membrane properties (Cappelen-Smith et al., 2001; Nodera et al., 2004). In multifocal motor neuropathy, ERC analysis by Kiernan et al. leads to the hypothesis that focal axonal depolarization at the conduction block is accompanied by axonal hyperpolarization below the block (Kiernan et al., 2002). Indeed, ERC studies can provide information on membrane polarization. If the axon is depolarized, the refractory period increases and the supernormal period decreases. If the axon is hyperpolarized, changes are in the opposite direction (Kiernan and Bostock, 2000). By using ischemia maneuvers, it is possible to replicate ERC modifications depending on membrane polarization. During ischemia, inactivation of Na^+/K^+ ATPase pumps produces membrane depolarization. After ischemia, there is an overactivation of these pumps responsible of membrane hyperpolarization.

In routine practice, electroneuromyography (ENMG) allows for the diagnosis and monitoring of peripheral neuropathies but does not provide information on changes in axonal excitability. Currently, TT techniques, which allow for the measurement of multiple excitability parameters with a semi-automated protocol called TROND (Trondheim) protocol, are internationally recognized as the gold standard for peripheral axonal excitability (Bostock et al., 1998). However, these techniques require the purchase of specialized equipment and software dedicated to these measurements, as well as a license renewal. Therefore, the TT procedure is not currently used in most clinical electrophysiology laboratories.

This prospective study was undertaken to demonstrate the feasibility and reliability of conducting ERC measurements with a conventional electrodiagnostic (EDX) device commonly used in routine clinical neurophysiology. To do so, we performed ERC measurements using the paired pulses' technique on a conventional EDX machine (CEM) and with the reference TT method. The study was conducted under basal conditions, and during and after ischemia maneuvers on forty healthy volunteers and on ten patients with CMT1A.

2. Material and methods

2.1. Population

Forty consecutive healthy volunteers (mean age = 41 ± 14 years old; range 23–66), including 23 men and 17 women and ten patients with CMT1A (mean age = 42 ± 18 years old; range 24–79) benefited from a prospective evaluation of the ERC. Healthy subjects belonged to medical or paramedical staff and their family or friends. None had clinical or electrophysiological signs of diffuse or localized peripheral neurological involvement, including carpal tunnel syndrome, and none had the usual risk factors for peripheral neuropathy (diabetes, alcohol or neurotoxic drug abuse). Patients with CMT1A had all a duplication of the PMP22 (Peripheral Myelin Protein 22) gene on genetic testing and were followed at our neuromuscular disease reference center. Test-retest and left–right reproducibility were assessed separately from the main control group in 15 and 16 healthy volunteers, respectively. The protocol was approved by hospital-faculty ethics committee of CHU Liege (B7072022000001). Written informed consent was obtained from all participants.

2.2. Recording and stimulating settings

The median nerve innervated thenar muscles was studied with classical motor nerve conduction settings. The ground, recording and stimulating electrodes consisted of pre-gelled disposable surface electrodes (Spes Medica Srl, DENIB05026).

Regarding the excitability parameters, the recording electrode (E1) was placed over the thenar eminence in close proximity to the muscle endplates halfway between the midpoint of the distal wrist crease and the first metacarpophalangeal joint. The reference electrode (E2) was placed over the dorsum of the proximal phalanx of the thumb. The ground electrode (E0) was placed over the ventral part of the forearm. The cathode was placed 2.5 cm proximal from the distal wrist crease along the course of the median nerve, and the anode 8 cm proximal from the cathode on the radial forearm. Skin impedances under the cathode, anode and ground electrode were systematically measured and kept near 5 kΩ or less by gently rubbing the skin with a sandpaper, cleaning it with alcohol and rubbing it again with an abrasive and conductive paste. The wrist temperature was maintained above 31 °C using a heating splint specially made for the study of nerve excitability (Tyberghein et al., 2023). This setup was positioned only once for both techniques.

Regarding the classic EDX parameters, motor distal latency, motor conduction velocity, and compound muscle action potential (CMAP) amplitude, the surface recording setting was identical. Stimulation was applied by a bipolar surface stimulation with two 7 mm diameter felt tip pads, 2.3 cm apart (Natus Medical Incorporated, REF 9013L0362), at the wrist and elbow.

2.3. Paired pulses' procedure with CEM

For the CEM procedure, data were collected using a Keypoint G3 ENMG machine (Natus Medical Incorporated, Keypoint.net software). The bandpass filter setting was set from 20 to 5000 Hz. The complex burst stimulation mode was used to apply two stimuli successively. This mode allowed separate adjustment of the intensity of the 1st and 2nd stimulus and modification of the inter-stimuli interval (ISI). The stimulus duration was 0.2 ms. The intensity of the first stimulus (conditioning stimulus) was determined to produce a maximal CMAP. The intensity of the test stimulus was adjusted such that for an ISI of 400 ms (ISI for which the test stimulus was no longer influenced by the conditioning stimulus), the amplitude of the evoked motor response corresponded to 40% of the maximal CMAP. Once the shocks intensities were determined, the ISI was gradually reduced to study 27 different intervals (400, 300, 200, 150, 100, 80, 60, 40, 30, 20, 15, 10, 7, 6, 5, 4, 3.75, 3.5, 3.25, 3, 2.75, 2.5, 2.25, 2, 1.75, 1.5 and 1 ms). A final paired pulse with an ISI of 400 ms was applied to evaluate the stability of the response. If the difference in response amplitude to the test stimulus was greater than 30% between the first and last paired pulses, the ERC study was restarted. The variation in the amplitude of the response to the test stimulus was measured for each ISI

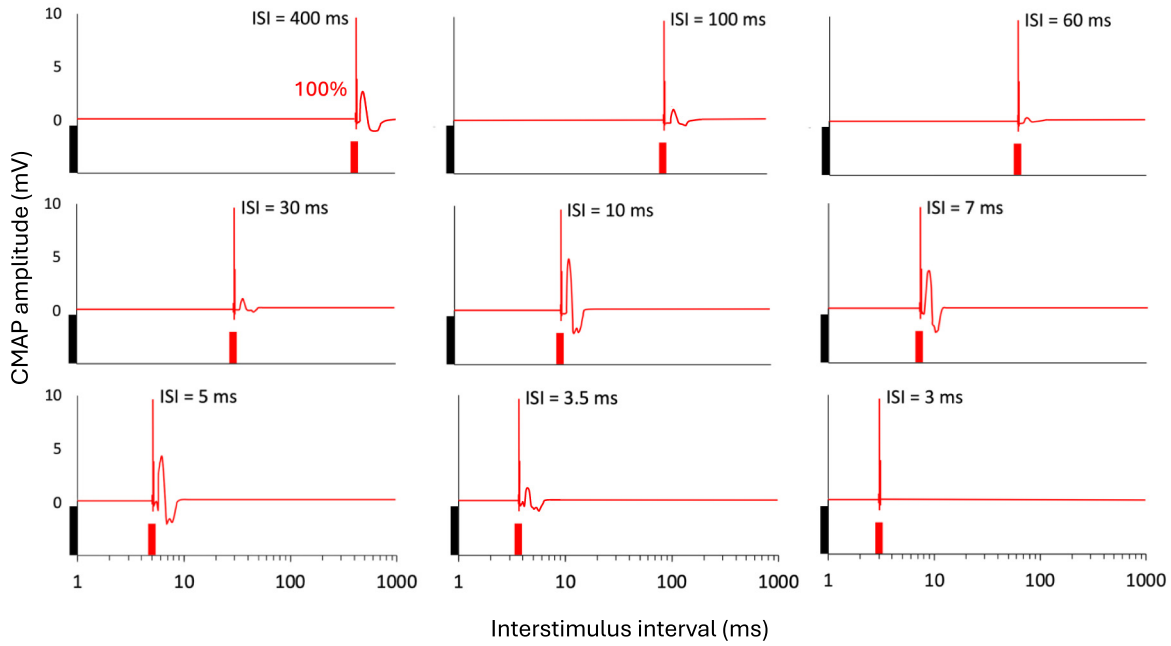

Fig. 1. Paired pulses' procedure using a conventional electrodiagnostic machine (CEM technique) in a healthy control subject. Two stimuli, one supramaximal conditioning (not visible in the figure, but its intensity is represented by the black rectangle) and the other test (whose intensity is represented by a red rectangle) are delivered with a decreasing inter-stimulus interval (ISI) from 400 ms to 1 ms (27 ISI in total, 9 of which are illustrated: 400, 100, 60, 30, 10, 7, 5, 3.5, and 3 ms). The intensity of the test stimulus is adjusted such that for an ISI of 400 ms (ISI for which the test stimulus is no longer influenced by the conditioning stimulus), the amplitude of the evoked motor response corresponds to 40% of the maximal compound muscle action potential (CMAP). As the ISI decreases, initially, the response amplitude decreases during the late subexcitable period, which is maximal around 60 ms. Then, as the ISI decreases further, the amplitude of the response increases during the supernormal period, which is maximal around 10 ms. Subsequently, the amplitude decreases again during the relative refractory period until there is no longer a response to the test stimulus (ISI = 3 ms in this example), which corresponds to the maximal absolute refractory period. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 1). When the ISI was less than 15 ms, the two responses (conditioned and tested) overlapped. To reconstruct the response to the test stimulus, a trace with the conditioning test alone was subtracted from each trace with an ISI less than 15 ms.

In results section of this work, the amplitude of all responses evoked by paired pulses at different ISI was always normalized (and expressed in %) relative to the response evoked by the test stimulus when the ISI was 400 ms.

This procedure was realized three times, in basal condition, after 5 min of ischemia and 3 min after ischemia (post ischemia). The ischemia was produced with an armband sphygmomanometer keeping pressure above 200 mm Hg.

The time required to determine the supramaximal conditioning stimulus and the stimulus test (ISI = 400 ms) was 2 min and 30 s. The recording time for the 27 ISI also took 2 min and 30 s. The analysis time to establish each normalized amplitude (in an Excel sheet) and to represent the ERC curve was about 10 min. Thus, it took 15 min to finalize an ERC analysis in any of the 3 conditions, see the Supplementary Video.

The excitability parameters studied are described in Fig. 2A. The percentage of subexcitability and superexcitability were the mean of the three lowest values around 40 ms after the conditioning stimulus and the three highest values around 7 ms after the conditioning stimulus, respectively. ARP corresponded to the maximum ISI for which there was no response to the test stimulus. RRP was the minimum ISI for which the normalized amplitude to test stimulus reached 100%. Area SUP was the area under the curve during the supernormal period and area SUB was the area over the curve during late subnormal period. The area SUP and area SUB were measured by summing up all the areas of trapezoids formed, between each ISI, by the ERC curve and the 100% line.

The validation process of the CEM involved a study conducted on 16 healthy volunteers to compare responses from the left and the right sides in each subject. To assess reproducibility, another study was conducted on 15 healthy volunteers comparing responses obtained from each subject on two occasions, with at least one week apart.

2.4. Threshold tracking procedure

TT procedure was conducted semi-automatically following the methodology described by Kiernan et al. (2000). TT techniques involved measuring the intensity (referred to as threshold) required to reach the target response, set at 40% of maximal CMAP amplitude. Unlike the CEM, the intensity required to maintain the stable target response was measured. First, the intensity required to elicit a maximal CMAP was assessed. Then, the stimulus–response curve was recorded to set the target response (40%). To study the ERC, 18 ISI were recorded (200, 140, 100, 75, 56, 42, 32, 24, 18, 13, 10, 7.9, 6.3, 5, 4, 3.2, 2.5, 2 ms). Three stimulus combinations were tested sequentially: (1) unconditioned test stimulus (1 ms duration) tracking the control threshold; (2) maximal conditioning stimulus (1 ms duration) alone; and (3) conditioning + test stimuli. The response to (2) was subtracted online from the response to (3) before measuring the CMAP test, ensuring that the maximal conditioning CMAP did not contaminate the measured response when the conditioning–test interval was short. Each stimulus combination was repeated until four valid threshold estimates were obtained. The results were expressed, for each ISI, as a threshold change expressed as a percentage relative to the control threshold when applying a test stimulus alone.

The excitability parameters studied are described in Fig. 2B. The percentage of subexcitability and superexcitability were the mean of the three highest values around 40 ms after the conditioning stimulus and three lowest values around 7 ms after the condition-

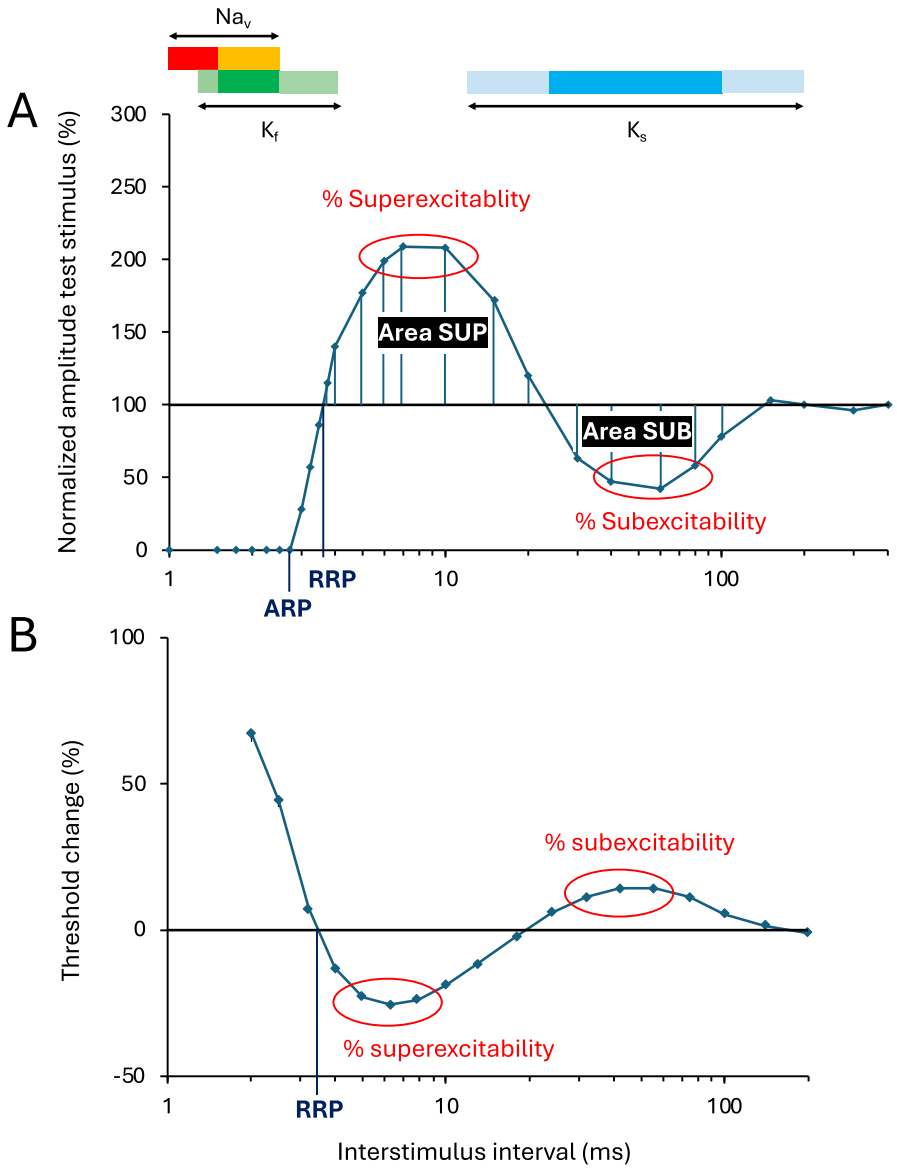

Fig. 2. Median (blue curves) of the results of nerve excitability recovery cycle (ERC) obtained in 40 healthy volunteers. A) using a technique with a conventional electrodiagnostic device (CEM technique); B) using the threshold tracking technique (TT technique). All responses to the 27 (A) and 18 (B) inter-stimulus intervals are normalized (%) relative to the response obtained by the test stimulus delivered alone (B) or by a paired pulses with an interval inter-stimulus of 400 ms at the beginning of the recording (A). The horizontal black lines represent the normalization reference for the different responses. With the CEM technique (A), it is the amplitude of the responses that is normalized, with the TT technique (B), it is the threshold reflecting the stimulation intensity that is normalized. The percentage of superexcitability and subexcitability are calculated by averaging the three extreme values recorded during the early supernormal period (around 7 ms) and the late subnormal period (around 40 ms). ARP = absolute refractory period, RRP = relative refractory period, Area SUP = area under the curve during the superexcitable period, Area SUB = area over the curve during the late subexcitable period. The areas are calculated by adding the trapezoidal surfaces delimited by the blue vertical line, the horizontal black line, and the ERC curve. At the top of the figure, the channels involved are schematically represented based on theoretical physiological knowledge, in relation to the x-axis (time in ms): nodal voltage-gated sodium channel (inactivated in red, recovery from inactivation in orange); fast potassium channel opening (in green) and slow potassium channel opening (in blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ing stimulus, respectively. RRP was the minimal value for which the threshold change was 0%.

2.5. Statistical analysis

Collected data included age, gender, height, weight, calculated body-mass index (BMI), and excitability measurements (absolute and relative refractory period durations, supernormal and subnormal periods expressed as a percentage amplitude or an area value). The majority of the statistical analysis was performed by using SAS software (SAS University Edition, Cary, NC). Descriptive statistics were expressed as means and standard deviations in control group if variables were distributed normally or as medians and interquartile ranges (IQR) in the small size CMT1A group (n = 10) and if variables were not distributed normally. Comparisons between control and CMT1A groups were performed using non-parametric Wilcoxon signed-rank test. Comparisons between basal condition, ischemia and post-ischemia were done using paired Student t-test in control group and paired Wilcoxon test in CMT1A group. Correlations between variables were tested by the Pearson correlation coefficient (r_p) or the non-parametric Spearman correlation coefficient (r_s) depending on whether the distribution of the variable was normal or not, respectively. Intra-class correlation coefficients (ICC) between test and retest and receiving operator

characteristic (ROC) with area under the curve (AUC), to compare the diagnostic performance of the two studied techniques, were analysed by the JASP software (version 0.18.1, copyright 2013–2023 University of Amsterdam). A two-sided p value <0.05 was considered significative. The limits of normal for excitability parameters were established in the control group by the percentile method (P5–P95).

3. Results

3.1. Clinical characteristics of the population

In the Table 1, we compared clinical and EDX characteristics of healthy volunteers and patients with CMT1A. No significant difference was observed between the two groups ($p > 0.05$) for all clinical data (age, height, weight, body mass index and gender). Regarding median nerve conduction studies, significant differences were observed between the two groups. In CMT1A group compared to the control group, motor amplitude and motor conduction velocity in the forearm were significantly lower and motor distal latency was higher.

3.2. Results in healthy volunteers in basal conditions

With CEM procedure, median ARP was 2.70 ms, mean RRP was 3.61 ms, mean percentage of superexcitability was 203.5% and mean percentage of subexcitability was 45.6% (Table 2, Fig. 2A). With TT technique, mean RRP was 3.51 ms, mean percentage of superexcitability was −25.0% and mean percentage of subexcitability was 14.3% (Fig. 2B). Specifically, regarding the CEM technique, the mean area SUP was 1,290%.ms and that of mean area SUB was 4,429%.ms.

All studied excitability parameters were independent of weight, height and BMI. There was a slight but significant correlation between age and percentage of superexcitability with CEM ($r_p = -0.505$; $p = 0.0009$) and TT ($r_p = 0.449$; $p = 0.0037$), between age and area SUP with CEM ($r_p = -0.523$; $p = 0.0005$) and between gender and percentage of superexcitability with TT ($r_p = -0.329$; $p = 0.0379$), superexcitability being increased in women (mean for women = −27.3% versus mean for men = −23.3%; Student t-test p value = 0,0379) (Table 3).

3.3. Reliability of the paired pulses' technique with CEM

One of the difficulties of the CEM method is to ensure stable experimental conditions during the application of the 27 paired pulses with ISI from 400 to 1 ms. To verify this stability, the motor response amplitude to test stimulus between initial and ultimate paired pulses, both with an ISI of 400 ms were compared. If the difference in amplitude between the two motor responses was greater than 30%, the CEM procedure was restarted (11% of cases in basal conditions). Ultimately, among the data retained for this study, the mean difference between the two test responses obtained by a paired pulse with an ISI of 400 ms at the beginning and end of the evaluation was 4% (±14%).

In this study, the TT technique was considered as the gold standard. To assess the external validity of the CEM technique, correlations between the data derived by both techniques were evaluated, and their respective diagnostic performances were assessed using ROC curves and AUC. There were statistically significant correlations between the two procedures, in healthy volunteers, concerning the percentage of superexcitability ($r_p = -0.592$, $p < 0.0001$), the percentage of subexcitability ($r_p = -0.559$, $p = 0.0002$) and the relative refractory period ($r_p = 0.559$, $p = 0.0002$) (Table 3). ROC curves were generated to compare the diagnostic performance of excitability parameters measured by both techniques. These ROC curves were constructed from data of healthy volunteer versus CMT1A patients (Table 4). In this study, the diagnostic performance of the methods under investigation was considered excellent when the AUC was greater than 0.90. The highest AUC was obtained for the area SUPCEM parameter (0.98). The AUC of the percentage of superexcitability was equivalent for both techniques (0.94 with TT and 0.95 with CEM).

To assess fidelity of the CEM, excitability parameters were compared between two tests, at least one week apart, in 15 healthy volunteers using intraclass correlation (ICC) (Table 5). The repeatability was particularly good for ARP (ICC = 0.80), the percentage of superexcitability (ICC = 0.76) and area SUPCEM (ICC = 0.78). The Spearman correlation comparing normalized amplitude responses to test stimulus for each ISI and for each subject was high ($r_s = 0.93$, $p < 0.0001$) (Fig. 3CD).

To support the fidelity, we also compared normalized amplitude responses to test stimulus for each ISI and for each subject between the left and right sides in 16 healthy volunteers (Fig. 3AB). The Spearman correlation was high ($r_s = 0.96$, $p < 0.0001$).

3.4. Results in healthy volunteers during ischemia maneuvers

We compared the results in healthy volunteers in the basal condition, during ischemia and in the post-ischemia (Table 2 and Fig. 4A). The statistically significant changes were observed: (1) during ischemia, there was an increase in refractory periods (ARP and RRP), a decrease in superexcitability (percentage of superexcitability and area SUP) and an increase in late subexcitability (percentage of subexcitability); (2) during post-ischemia, there was a decrease in refractory period (ARP and RRP), an increase in superexcitability (percentage of superexcitability and area SUP) and a decrease in late subexcitability (percentage of subexcitability and area SUB).

The correlation analysis revealed a negative correlation between the area SUP and area SUB during the post-ischemic period ($r_p = -0.549$, $p = 0.0002$) (Table 3).

3.5. Comparison of healthy volunteers and patients with CMT1A disease in basal condition

We compared results in healthy volunteers and patients with CMT1A in basal conditions using both techniques, TT and CEM (Table 4, Fig. 4CD). Whatever the technique, the superexcitable period was significantly decreased in CMT1A compared to healthy volunteers (percentage of superexcitability with CEM and TT, area

Table 1
Clinical and EDX characteristics (median and IQR) of healthy volunteers (control) and patients with Charcot-Marie-Tooth type 1A disease (CMT1A).

	Control (n = 40)	CMT1A (n = 10)	Wilcoxon p value
Age (year)	38.5 (26.5)	36.0 (18.0)	1.00
Height (cm)	1.76 (0.13)	1.70 (0.10)	0.12
Weight (kg)	71.0 (14.0)	65.5 (29.0)	1.00
Body mass index (kg/m^2)	23.0 (3.5)	24.5 (9.0)	0.37
Gender	42.5*	60.0*	0.33
Median nerveCMAP amplitude (mV)	8.90 (2.70)	4.8 (1.53)	<0.0001
Median nerve distalCMAP latency (ms)	3.84 (0.40)	9.00 (1.20)	<0.0001
Median nerve forearmMCV (m/s)	56.2 (4.20)	24.6 (2.63)	<0.0001

EDX = electrodiagnostic; IQR = interquartile range; CMAP = compound muscle action potential; MCV = motor conduction velocity.
*% female.

Table 2
ERC parameters evaluated by a conventional electrodiagnosic machine in healthy volunteers in basal condition (BC), ischemia (Isch) and post-ischemia (P-Isch), mean value (standard deviation).

	BC (n = 40)	Isch (n = 39)	P-Isch (n = 40)	Paired comparisons		
				BC/Isch p value	BC/P-Isch p value	Isch/P-Isch p value
ARP (ms)*	2.70 (0.50)	3.70 (0.50)	2.20 (0.70)	<0.0001	<0.0001	<0.0001
RRP (ms)	3.61 (0.55)	6.39 (1.27)**	3.08 (0.50)	<0.0001	<0.0001	<0.0001
Superexcitability (%)	203.5 (28.6)	98.5 (28.8)	229.8 (25.6)	<0.0001	<0.0001	<0.0001
Subexcitability (%)	45.6 (20.8)	38.4 (16.9)	65.8 (26.4)	0.0137	<0.0001	<0.0001
Area SUP (%.ms)*	1,290 (585)	1.5 (125)	3,284 (2,306)	<0.0001	<0.0001	<0.0001
Area SUB (%.ms)	4,429 (2,514)	4,195 (1,984)	3,489 (2,347)	>0.05	0.0133	>0.05

ERC = excitability recovery cycle; IQR = interquartile range; ARP = Absolute refractory period; RRP = relative refractory period; Area SUP = area under the curve during superexcitable period; Area SUB = area over the curve during late subexcitable period.
* Median (IQR) and non-parametric statistic (variable not distributed normally).
** n = 27.

Table 3
Summary of statistically significant Pearson correlations in the control group (n = 40).

		r_p	p value
Age (year)	Superexcitability $_{CEM}$ (%)	−0.505	0.0009
Age (year)	Superexcitability $_{TT}$ (%)	0.449	0.0037
Age (year)	Area SUP $_{CEM}$ (%)	−0.523	0.0005
Gender (F/M)	Superexcitability $_{TT}$ (%)	−0.329	0.0379
RRP $_{CEM}$ (ms)	RRP $_{TT}$ (ms)	0.559	0.0002
Superexcitability $_{CEM}$ (%)	Superexcitability $_{TT}$ (%)	−0.592	<0.0001
Subexcitability $_{CEM}$ (%)	Subexcitability $_{TT}$ (%)	−0.559	0.0002
Area SUP $_{CEM}$ (%.ms) (during post-ischemia)	Area SUB $_{CEM}$ (%.ms) (during post-ischemia)	−0.549	0.0002

CEM = conventional electrodiagnostic machine technique; TT = threshold tracking method; RRP = relative refractory period; Area SUP = area under the curve during superexcitable period; Area SUB = area over the curve during late subexcitable period; r_p = Pearson correlation coefficient.

SUP). With the CEM procedure, the response amplitude to the test stimulus increased up to 208% in controls versus 143% in CMT1A. The ARP with the CEM technique and the RRP with the TT technique were significantly decreased in patient with CMT1A. There was no significant difference in the late subexcitable period with the TT technique, whereas it was decreased in CMT1A using the CEM technique (percentage of subexcitability and area SUB).

3.6. Results in patients with Charcot-Marie-Tooth type 1A disease during ischemia maneuvers

In patients with CMT1A, the percentage of superexcitability significantly decreased during ischemia and increased during post-ischemia, but the changes were less highly significant than in healthy volunteers. There was no significant variation in the late subexcitability period (percentage of subexcitability and area SUB) between the three conditions. The ARP significantly increased during ischemia, while there was no significant change for the RRP (Table 6, Fig. 4B).

4. Discussion

Many techniques, often complementary, exist to study axonal excitability ranging from the very ancient measurements of chronaxie and rheobase (Lapicque and Lapicque, 1903; Weiss, 1901) to the highly sophisticated measurements allowed by threshold tracking (Bostock et al., 1998), including the simple measurement of the minimal intensity required to obtain a maximal CMAP (Parker et al., 2016; Tyberghein et al., 2022). These techniques, which are not usually used in routine practice, have contributed to extending the field of electrophysiology exploration ever further. In this work, we continue our efforts to enable broader access to techniques for evaluating peripheral nerve excitability. After iMAX (Tyberghein et al., 2022) and measurements of the strength-duration time constant using a manual method (Tyberghein et al., 2023), we are interested in studying ERC using conventional EDX equipment. The study of axonal excitability changes following the passage of an action potential can give information about the proper functioning of the axonal membrane and ion channels. Indeed, refractory periods depend on inactivation of transient Na^+ channels. The superexcitable period reflects the presence of a depolarizing afterpotential limited by the activation of fast K^+ channels. As for the subexcitable period, it depends on activation of slow K^+ channels (Fig. 2). Alteration of the number or the

Table 4
ERC parameters (median and IQR values) evaluated by a conventional electrodiagnosic machine (CEM) and the threshold tracking (TT) techniques in healthy volunteers (Control) and Charcot-Marie-Tooth disease type 1A (CMT1A).

	Control (n = 40)	CMT1A (n = 10)	Wilcoxon p value	ROC curves	
				AUC	Cut off values
ARP $_{CEM}$ (ms)	2.70 (0.50)	1.60 (0.50)	<0.0001	**0.94**	2.2
RRP $_{CEM}$ (ms)	3.60 (0.75)	3.05 (1.30)*	NS	0.64	2.5
Superexcitability $_{CEM}$ (%)	208.0 (27.0)	143.5 (65.0)	<0.0001	**0.95**	156
Subexcitability $_{CEM}$ (%)	45.5 (30.5)	68.5 (25.0)	0.0032	0.81	81
Area SUP $_{CEM}$ (%.ms)	1,290 (585)	303 (439)	<0.0001	**0.98**	478
Area SUB $_{CEM}$ (%.ms)	4,146 (3,753)	1,961 (1,937)	0.0015	0.82	1,418
RRP $_{TT}$ (ms)	3.55 (0.65)	3.00 (0.40)	0.0216	0.74	2.7
Superexcitability $_{TT}$ (%)	−26.0 (8.0)	−10.3 (9.5)	<0.0001	**0.94**	−15
Subexcitability $_{TT}$ (%)	14.0 (4.0)	11.1 (7.1)	NS	0.68	5

ERC = excitability recovery cycle; IQR = interquartile range; ROC = receiver operating characteristic; AUC = area under the curve; ARP = Absolute refractory period; RRP = relative refractory period; Area SUP = area under the curve during superexcitable period; Area SUB = area over the curve during late subexcitable period; *n = 8. Excellent diagnostic performance was assumed when AUC >0.90 (in bold).

Table 5
Test-retest repeatability of conventional electrodiagnosic machine technique (n = 15).

	Test median (IQR)	Retest median (IQR)	ICC
ARP (ms)	2.50 (0.50)	2.50 (0.76)	**0.80**
RRP (ms)	3.50 (0.63)	3.15 (0.47	0.07
% superexcitability	217.6 (21.1)	219.3 (14.0)	**0.76**
% subexcitability	35.5 (14.4)	31.5 (14.4)	0.70
Area SUP (%.ms)	1,486 (377)	1,424 (291)	**0.78**
Area SUB (%.ms)	4,992 (1,554)	5,197 (2,509)	0.43

ICC = intraclass correlation coefficient; ARP = absolute refractory period; RRP = relative refractory period; Area SUP = area under the curve during superexcitable period; Area SUB = area over the curve during late subexcitable period. Good repeatability was assumed when ICC >0.75 (in bold).

distribution and density of ion channel by demyelination or axonal destruction (Cappelen-Smith et al., 2001), changes in membrane polarity (Kiernan and Bostock, 2000), alterations in ion concentration (Kuwabara et al., 2007), are all factors that can modify excitability cycle.

The study of ERC was conducted in three conditions (baseline, ischemia, and post-ischemia) using the CEM technique in a control group (n = 40) and a CMT1A group (n = 10). In both groups, ERC analysis was also performed using the TT method. The two groups did not differ significantly statistically in terms of age, height, weight, BMI, and gender. However, the CMT1A group exhibited EDX parameters characteristic of demyelinating neuropathy, which distinguished it significantly from the control group (Table 1).

The excitability parameters studied were the percentage of superexcitability, the percentage of subexcitability, and RRP with both techniques. With the CEM technique we were able to study three additional parameters: (1) ARP (maximal ISI for which there was no response to the test stimulus); (2) the area under the curve during superexcitability (area SUP); (3) the area over the curve during late subexcitability (area SUB). The diagnostic performance of these different parameters to correctly identify the two groups in terms of sensibility and specificity was measured using ROC curves. The highest AUC were measured in descending order with area SUP (0.98), the percentage of superexcitability with CEM (0.95) and TT (0.94), and with ARP (0.94) (Table 4). Furthermore, in the test–retest study conducted in a control group (n = 15), the ICC for area SUP was found to be good (0.78) (Table 5). The area SUP parameter appears to be particularly relevant for assessing the early supernormal period in ERC study.

The correlation analysis between excitability data and the physical and demographic characteristics of healthy volunteers revealed a significant reduction in parameters measuring the early supernormal period (percentage of superexcitability with CEM and TT, area SUP with CEM) with age (Table 3). Aging could be responsible for a decrease in the capacity of the internodal axolemma to store electric charges and to generate an increase of excitability after the action potential. In the perspective of using ERC study for diagnostic purposes, this negative correlation with age should be taken into account by integrating this into a predictive analysis such as linear regression. The correlation analysis also showed a significant relationship between the percentage of superexcitability with TT and gender, with women showing significantly higher values (p = 0.0379). These modifications in excitability with age and gender, were not described in other studies (Borg, 1980; Caetano et al., 2022; Casanova et al., 2014). Conversely, ERC and especially the refractory periods were influenced by temperature (Boërio et al., 2004). To overcome this issue in this study, the nerve temperature was maintained stable, above 31°, throughout the test using a heating splint (Tyberghein et al., 2023).

The reliability of paired pulses technique with CEM was assessed in healthy volunteers in four different ways: (1) fidelity was evaluated through a test–retest study (n = 15) and incidentally by comparing the results obtained for the left and right hands (n = 16); (2) external validity was established by comparing CEM data with those obtained using the TT technique, considered as the reference method (n = 40); (3) stability during the test was verified by comparing, for each control subject, the first and last responses to the test stimulus, both with an ISI of 400 ms (n = 40); (4) the results obtained in the three conditions (baseline,

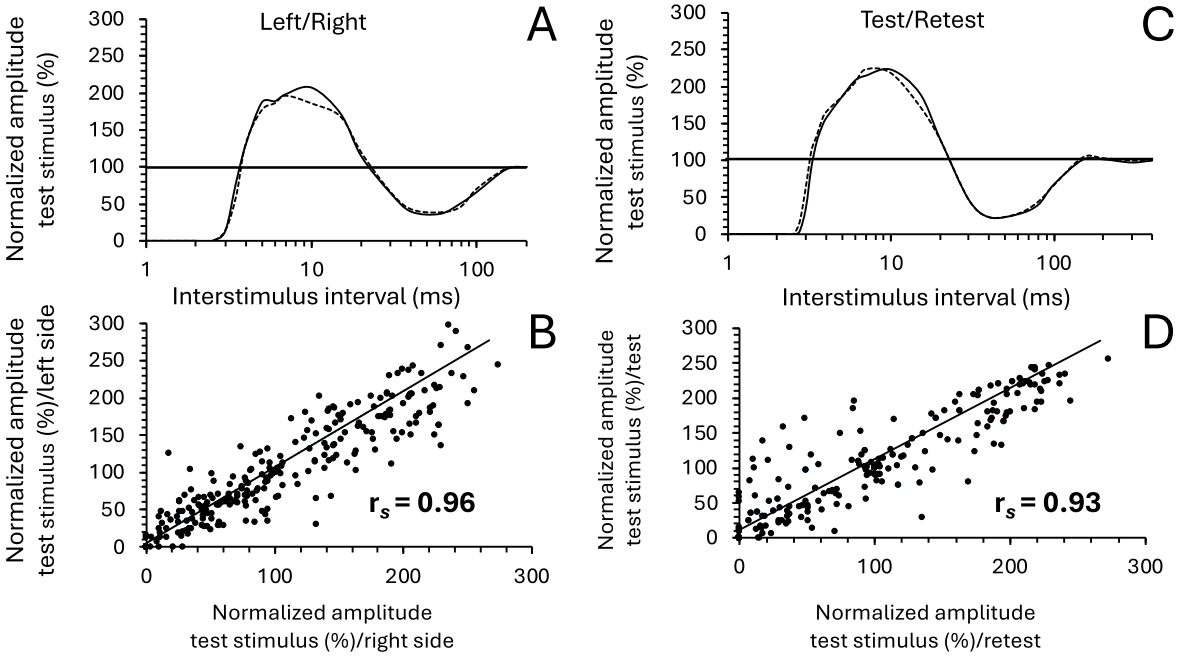

Fig. 3. Excitability recovery cycle study with the conventional electrodiagnostic machine. In A and B, comparison of right side (black continuous line) and left side (dotted line) in 16 healthy volunteers. In C and D, comparison of first test (black continuous line) and second test (dotted line) one month apart in 15 healthy volunteers. A and C: Median normalized response amplitude to the test stimulus according to inter-stimulus interval. B and D: Spearman correlation analysis (r_s = Spearman correlation coefficient) comparing normalized amplitude response to test stimulus for each inter-stimulus interval and for each subject.

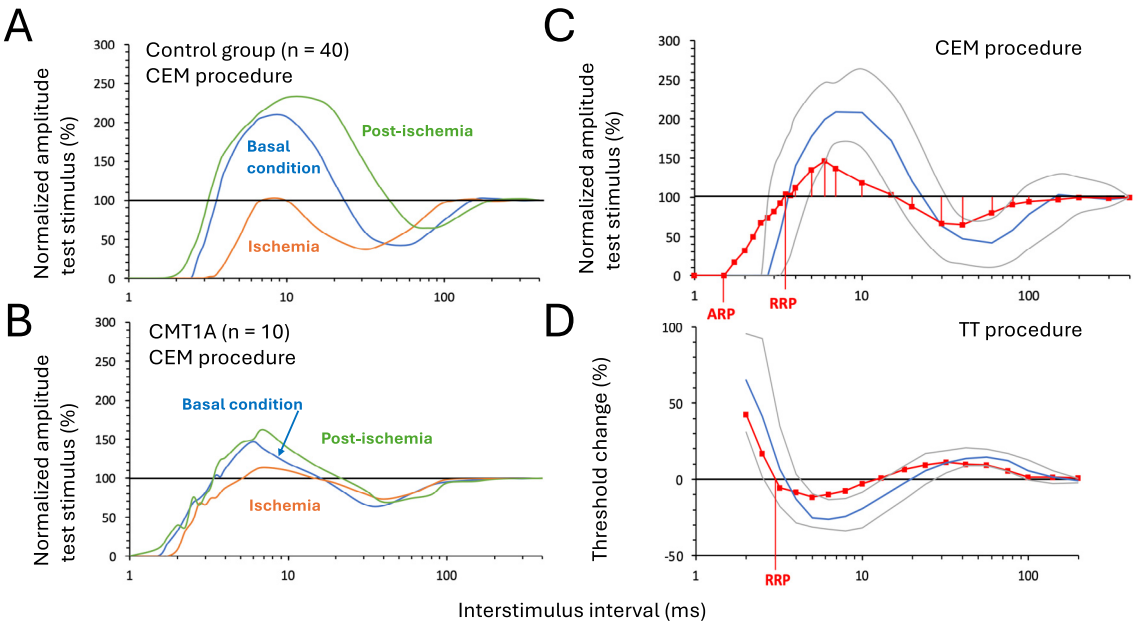

Fig. 4. A, B, C) Results of excitability recovery cycle (ERC) with the conventional electrodiagnostic machine (CEM); D) with the threshold tracking procedure (TT). A, B) Results during ischemic and post-ischemic maneuvers (blue = basal condition, orange = ischemia, green = post-ischemia); (A) In control group, during ischemia, the refractory period is increased, superexcitability is decreased and late subexcitability is increased, and during post-ischemia, the refractory period is decreased, superexcitability is increased and late subexcitability is decreased (these ERC variations are all statistically significant); (B) In Charcot-Marie-Tooth disease type 1A (CMT1A), ERC variations during ischemia and post-ischemia are similar to those in the control group but less pronounced. (C, D) Results in the control group (n = 40; median in blue; P5 and P95 in grey) and in CMT1A (n = 10; median in red). ARP = absolute refractory period, RRP = relative refractory period. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 6
ERC parameters (median and IQR values) evaluated with a conventional electrodiagnosic machine in patients with Charcot-Marie-Tooth disease type 1A (CMT1A) in basal condition (BC), ischemia (Isch) and post-ischemia (P-Isch).

CMT1A (n = 10)	BC	Isch	P-Isch	Paired comparisons		
				BC/Isch p value	BC/P-Isch p value	Isch/P-Isch p value
ARP (ms)	1.60 (0.50)	1.85 (1.50)	1.25 (0.70)	0.0469	>0.05	0.0156
RRP (ms)	3.05 (1.30)*	4.50 (1.20)**	3.00 (1.30)***	>0.05	>0.05	>0.05
Superexcitability (%)	143.5 (65.0)	111.0 (29.0)	156.0 (53.0)	0.0254	0.0215	0.0137
Subexcitability (%)	68.5 (25.0)	78.0 (22.0)	71.5 (7.0)	>0.05	>0.05	>0.05
Area SUP (%.ms)	303 (439)	87 (123)	430 (530)	>0.05	>0.05	0.0195
Area SUB (%.ms)	3,164 (1,937)	1,256 (2,095)	2,405 (1,519)	>0.05	>0.05	>0.05

ERC = excitability recovery cycle; IQR = interquartile range; ARP = Absolute refractory period; RRP = relative refractory period;
Area SUP = area under the curve during superexcitable period; Area SUB = area over the curve during late subexcitable period;
* n = 8.
** n = 7.
*** n = 9.

ischemia, and post-ischemia) were compared to those from the literature (n = 40).

The ICC values between test and retest (Table 5) suggested good repeatability for ARP (0.80), percentage of superexcitability (0.76), and area SUP (0.78). The least reproducible parameter was RRP (ICC = 0.07). The dependence of the short RRP to the relatively long supernormal period could be responsible of the poor reproducibility of this value. Indeed, the periods of the recovery cycle overlap, such that changes in one period can affect size of previous and following periods (Kiernan et al., 2020). In the study of the reproducibility of TT parameters, RRP measurement was also found to be poorly reproducible (Pia et al., 2023). The fidelity of the CEM technique was further supported by the very high correlations observed for all parameters of the ERC measured at 27 ISI, both during test and retest (n = 15; r_s = 0.93; p < 0.0001), and during left/right comparison (n = 16; r_s = 0.96; p < 0.0001) (Fig. 3BD).

Comparison of results obtained by both method under study, one of which was considered as the reference (TT), in the same population revealed that the CEM technique was a valid method for studying ERC. The three parameters common to both procedures were correlated (Table 3), either positively (RRP) or negatively (percentage of super excitability and percentage of subexcitability) depending on how the results were expressed in the two techniques. Despite this different data treatment, it appeared that the various phases of the ERC coincided (Fig. 2). Finally, as discussed earlier, the diagnostic performance of both techniques assessed by ROC curve analysis was similar, particularly for the percentage of superexcitability (AUC = 0.95 with CEM and 0.94 with TT). Of course, it is important to keep in mind that the use of threshold tracking involves several different measurements in addition to the recovery cycle, and that these measurements are necessary to provide comprehensive information about the biophysics of normal or disordered myelinated fibers.

Two advantages of the TT procedure were the use of a feedback system to maintain a constant target amplitude, and the comparison of the conditioned response to the unconditioned response

at each interstimulus interval (Bostock et al., 1998). This was not the case with the CEM method. With this procedure, while it was easy to maintain the intensity of the test stimulus constant, if the examination conditions changed (e.g., more flexed wrist, less relaxed patient, fluctuations in the threshold), we could not guarantee that, for the same ISI, the test stimulus always produced a motor response of constant amplitude. In CMT1A patients, where the threshold was several times higher than in control subjects, a relative fluctuation in the threshold in this demyelinating disorder could have significant effects on response amplitude. During the CEM method, the stability of the test conditions was only checked at the end of the examination, when the response obtained for an ISI of 400 ms had an amplitude close to that which was evoked at the beginning of the examination for the same ISI of 400 ms. In 11% of cases, the amplitude fluctuation exceeded 30%, and we repeated the cycle study. By proceeding in this manner, the amplitude stability of responses to the test stimulus was ensured between the first and last paired pulses both with an ISI of 400 ms (mean amplitude variability = 4 ± 14%).

Lastly, another argument that validates the CEM procedure for studying ERC is that the results obtained under ischemic and post-ischemic conditions are consistent with those reported in the literature (Gilliatt and Willison, 1963; Grosskreutz et al., 1999; Kiernan and Bostock, 2000). During ischemia, we observed a significant increase in the refractory period (prolongation of Na_v channel inactivation) and a significant reduction in the supernormal period (decrease in the driving force), which can be related to nodal axonal membrane depolarization. The effects were reversed in the post-ischemic phase, with a significant reduction in the refractory period, even compared to the baseline condition (reduction in Na_v channel inactivation time), and a significant increase in the supernormal period, even compared to the baseline condition (increase in driving force), which can be related to nodal axonal membrane hyperpolarization (Table 2, Fig. 4A). Furthermore, during the post-ischemic phase, while the supernormal period was significantly increased, we observed a negative correlation between the area SUP and the area SUB (Table 3). These results suggest, as previously indicated (Kiernan et al., 2020), that the periods of the ERC overlap and that changes in one of these periods (increase in the early supernormal period) affect the size of the other periods (decrease in the late subnormal period). During ischemia, a significant increase of the percent subexcitability, compared to baseline, was observed (Table 2, Fig. 4A). This increase might be partly due to the decrease of the supernormal period. Along the same lines, we had already mentioned in this discussion that the dependance of the short relative refractory period to the relatively large supernormal period could explain the poor reproducibility of the RRP parameter.

In CMT1A patients, compared to healthy volunteers, both the refractory and supernormal periods were significantly reduced whatever the technique used (Table 4, Fig. 4CD). These results are in accordance with another study investigating excitability using the TT technique in nine patients with CMT1A (Nodera et al., 2004). The same modifications of the ERC have also been described in patients with acquired chronic demyelinating neuropathies (Cappelen-Smith et al., 2001). The reason for the reduced refractoriness in CMT1A remains unclear. The refractory period is due to the inactivation of transient Na channels. Theoretically, demyelination could induce a prolongation of the refractory period because more driving current would be needed to depolarize the driven node due to current leakage and increased nodal capacity (Franssen and Straver, 2013). One hypothesis would be that since juxtaparanodal demyelination lead to exposure of juxtaparanodal fast K^+ channels (Franssen and Straver, 2013; Rosenbluth and Bobrowski-Khoury, 2014; Schwarz et al., 1991), a faster activation of these channels, which repolarize the node, would result in a faster recovery from inactivation of transient Na channels and thus a decrease in refractory periods. In X-linked dominant Charcot-Marie Tooth disease (CMTX), refractoriness and RRP was also decreased and mathematical modelling showed an increase in "nodal" fast K^+ current (Liang et al., 2014). Superexcitability is due to a depolarizing afterpotential, following impulse conduction (Barrett and Barrett, 1982). After the action potential, the passive properties (large capacitance) of the internodal axolemma enable the slow storage of electric charges and generation of afterpotentials. This period is limited by the opening of fast K^+ channels. Changes in passive membrane properties and exposure of fast K^+ channels caused by demyelination could be responsible for the reduction of superexcitability in patients with demyelinating neuropathies (Kiernan et al., 2020). A decrease in late subexcitability in CMT1A patients was highlighted only with the CEM technique. Late subexcitability is due to the activation of nodal slow K^+ channels by depolarization. Activation of these channels prevents extreme depolarization, especially during short-lasting repetitive firing (Franssen, 2019). Paranodal demyelination could decreased the concentration of slow K^+ channels in the node and thus decrease the hyperpolarizing afterpotential.

Regarding ERC variations during and after ischemia, the ERC modifications were comparable in CMT1A patients to those in the control group but less pronounced. Only the increase in ARP during ischemia compared to baseline, the reduction in superexcitability during ischemia followed by an increase in post-ischemia, and the increase in area SUP during post-ischemia compared to ischemia were statistically weakly significant (Table 6, Fig. 4B).

We wish to express reservations regarding our study. Firstly, the study of refractory periods with CEM or TT techniques in motor nerves did not allow for the exact measurement of the axonal refractory period. Indeed, the parameters used (ARP and RRP) are influenced by factors such as neuromuscular transmission time and muscular refractory period (Boërio et al., 2004). The results in healthy volunteers were in accordance with other studies studying neuromuscular refractory periods (Kopec et al., 1978). However, in studies using other techniques, as recording action potentials directly from the nerve, the refractory periods were shortest (Betts et al., 1976; Gilliatt and Willison, 1963). Tankisi et al. suggest another technique with TT device called recovery cycle supramaximal (RCSM) protocol to overcome these factors and study the only axonal refractory period (Tankisi et al., 2022). This reservation is ultimately akin to what could be made in EDX regarding motor distal latency. Just because it does not exclusively measure nerve conduction does not mean it is not useful in carpal tunnel syndrome. The highly significant changes for ARP and RRP during ischemia and post-ischemia phases in accordance with literature data (Table 2) prove that the validity of the CEM technique should not be questioned. Similarly, before dismissing the idea of a decrease in the refractory period in CMT1A, already reported elsewhere (Nodera et al., 2004), and attributing it to a methodological bias, further studies specifically targeting the refractory period should be conducted in populations of patients with demyelinating neuropathy. The second reservation concerns the measurement of the RRP using the CEM technique. In the absence of a supernormal period, as was sometimes observed in healthy subjects during ischemia and even in some CMT1A patients in baseline condition, the amplitude of the response to the test stimulus never exceeded the 100% ERC limit, making it impossible to accurately measure the RRP. In these situations, data regarding RRP were excluded from the study.

5. Conclusion

Study of the ERC with the paired pulses' technique in a CEM is possible and reliable. The study in CMT1A showed a decrease of

refractory periods and decrease of superexcitability. The most relevant parameter to discriminate CMT1A from the control group were the area under the curve during superexcitability. We encourage electrophysiologists to study ERC in patients with peripheral neuropathies with their EDX device to provide information on motor axonal excitability.

Author contribution

Maelle Tyberghein contributed to the development of the design of this study, data acquisition, and article writing.

Florence Manto contributed to data acquisition.

François Charles Wang is the promotor of this work and contributed to the development of the design of this study, data acquisition, and article writing.

Conflict of interest statement

None of the authors have potential conflicts of interest to be disclosed.

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Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.clinph.2024.09.009.

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