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iMAX: A new tool for assessment of motor axon excitability. A multicenter prospective study

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HIGHLIGHTS

- The within and between center variability shows good reliability of the iMAX procedure.
- iMAX is fast, non-invasive and measurable at any stimulation point without specific equipment.
- In patients with peripheral neuropathy, iMAX allows monitoring excitability changes of motor axon.

ABSTRACT

Objective: This study was undertaken to establish by a multicentric approach the reliability of a new technique evaluating motor axon excitability.
Methods: The minimal threshold, the lowest stimulus intensity allowing a maximal response by 1 mA increments (iUP) and then by 0.1 mA adjustments (iMAX) were prospectively derived from three nerves (median, ulnar, fibular) in four university centers (Liège, Marseille, Fraiture, Nice). iMAX procedure was applied in 28 healthy volunteers (twice) and 32 patients with Charcot-Marie-Tooth (CMT1a), chronic inflammatory demyelinating polyneuropathy (CIDP), Guillain-Barré syndrome (SGB) or axonal neuropathy.
Results: Healthy volunteers results were not significantly different between centers. Correlation coefficients between test and retest were moderate (> 0.5). Upper limits of normal were established using the 95th percentile. Comparison of volunteers and patient groups indicated significant increases in iMAX parameters especially for the CMT1a and CIDP groups. In CMT1a, iMAX abnormalities were homogeneous at the three stimulation sites, which was not the case for CIDP.
Conclusions: The iMAX procedure is reliable and allows the monitoring of motor axon excitability disorders.
Significance: The iMAX technique should prove useful to monitor motor axonal excitability in routine clinical practice as it is a fast, non-invasive procedure, easily applicable without specific software or devices.

1. Introduction

Based on conduction studies and electromyography, electrodiagnosis (EDX) is an essential technique for characterizing peripheral neuropathies and quantifying their degree of damage. However, while it is a common experience that in order to obtain a supramaximal response, especially in some demyelinating neuropathies, it is often necessary to increase the amount of current above normal, axonal excitability does not participate routinely to the diagnosis of peripheral neuropathies. Yet EDX history, early in the 20th century, began by neuronal excitability studies. Weiss and Lapicque described the first parameters to quantify nervous excitability, chronaxie and rheobase (Weiss, 1901; Lapicque and Lapicque, 1903). The rheobase is the estimated threshold current for a stimulus of infinitely long duration. The chronaxie is the min-

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imum stimulus duration for a current twice rheobase to stimulate a muscle. Later, studies on isolated nerve fibers measured the recovery cycle with a sequence of refractoriness (absolute refractory period and relative refractory period), supernormal excitability and subnormal excitability (Bergmans, 1970). The recovery of excitability after a single supramaximal conditioning stimulus can assess these parameters using paired pulse with varying interstimulus interval typically between 2 and 200 ms. The major determinant of the recovery from refractoriness is the recovery of Na^+ channels from inactivation. Supernormal period comes from "back-flow" of current from the internodal membrane. The amount of current stored in the internodal membrane varies with membrane potential as paranodal fast potassium channels are either opened or closed. Late subnormal period is due to slow potassium channels. A number of studies have demonstrated relative refractory period impairment in axonal neuropathies such as diabetic neuropathy (Tackmann and Lehmann, 1980) and alcoholic neuropathy (Alderson and Petajan, 1987) or in carpal tunnel syndrome (Gilliatt and Meer, 1990). Regarding demyelinating neuropathies as chronic inflammatory demyelinating polyneuropathy (CIDP) or multifocal motor neuropathy with persistent conduction blocks (MMN), only a few studies found an impairment of the recovery cycle and most of them stated that there is no alteration of the recovery cycle in these neuropathies (Reitter and Johannsen, 1982; Boërio et al., 2010). Another approach to assess nerve excitability is the stimulus–response curve (Brismar, 1985; Boërio et al., 2008). This method establishes the nerve threshold range curve from the relation between compound muscle action potential (CMAP) size and strength of the stimulus to the nerve. Brismar (1985) demonstrated the advantage of this technique in diabetic, uremic and entrapment neuropathies. Cappelen-Smith et al. (2001) used these curves to compare healthy subjects and patients with CIDP. They demonstrated that thresholds of intensity are increased in CIDP especially near i90 (intensity threshold required to obtain 90% of the maximal CMAP size). Moreover, two studies were conducted in patients with MMN and demonstrated an increase of the i100 (Takanori et al., 1996; Priori et al., 2002).

In recent years, most of publications about peripheral nerve excitability come from Bostock and Kiernan, pioneers of the so-called threshold tracking and threshold electrotonus (Bostock et al., 1998; Kiernan et al., 2020). The basic principle of threshold tracking techniques is to measure the strength of the stimulus required to produce a CMAP of a specified size (near 40% of the maximal CMAP size) termed the "threshold" in different experimental conditions. This method allows to indirectly examine membrane potential changes that occur during long (100–200 ms) subthreshold current pulses (threshold electrotonus), demonstrating properties of the internodal membrane.

To date, for different reasons (need of special equipment or software, time-consuming procedure), none of these methods is used in current practice. Therefore, we wanted to develop a novel and practical EDX technique called the iMAX to assess peripheral motor axon excitability in patients with peripheral neuropathies. Compared to the previously described techniques, our technique is innovative because it would make it possible to measure nerve excitability in few minutes with a simple EDX device and classic recording and stimulating electrode settings (Milants et al., 2017). This test could easily be achieved in routine clinical practice and, thus, facilitate its implementation and use for many diseases. We conducted a multicenter study in four university hospitals: *Centre Hospitalier Universitaire* (CHU) de Liège (Belgium), *Centre Neurologique et de Réadaptation Fonctionnelle* (CNRF) de Fraiture (Belgium), CHU Marseille (France) and CHU Nice (France). The first goal of this study was to appreciate reliability of our technique and to set standards. The second goal was to demonstrate that our technique was able to measure motor axon excitability impairment in peripheral neuropathies.

2. Methods

2.1. Study design

The study design consisted of an attempt to establish the reliability of the iMAX procedure in 5 steps:

1) assess repeatability by measuring test–retest reliability
2) evaluate the consistency of iMAX parameters between the 4 centers
3) establish normative values beyond which an axonal hypoexcitability would be detected and possibly below which a superexcitability could be evoked
4) verify that the iMAX procedure is able to detect changes in excitability encountered in patients with peripheral neuropathy
5) analyzed our results by putting them in perspective with those of the literature

2.2. Study participants

Each center (Liège, Fraiture, Marseille, Nice) had to recruit at least 6 healthy volunteers, 2 patients with an axonal neuropathy and 6 patients with a demyelinating neuropathy.

The healthy volunteers group had to include one woman and one man between 20 and 40 years old, one woman and one man between 41 and 60 years old and one woman and one man over 60 years old. They were tested twice at least two days apart. The results of the first measurement (test) had to be hidden for the second record (retest). The protocol was approved by the different French (*Comité de Protection des Personnes*, promotor: *Assistance Publique-Hôpitaux de Marseille*) and Belgian (B707201837055) ethics committees. Written informed consent was obtained from all participants.

The patient group with demyelinating neuropathies had to include at least 2 patients with CIDP, 2 patients with a Charcot-Marie-Tooth Disease Type 1A (CMT1A) and 2 patients with a demyelinating (AIDP: acute inflammatory demyelinating polyneuropathy) or an axonal form (AMAN: acute motor axonal neuropathy; AMSAN: acute motor and sensory axonal neuropathy) of Guillain-Barré syndrome (GBS). Patients with GBS had to be seen twice, once before day 14 from onset of symptoms and one after the day 28.

2.3. Electrophysiological protocol

Electrophysiological testing was performed in each center by the same examiner (4 centers/4 investigators) using a KEYPOINT® G4 (Marseille) or G3 (Liège, Fraiture, Nice) Workstation ENMG machine (Natus Medical Incorporated). We followed the protocol submitted by Milants et al. (2017). Prior to the study itself, two workshops were organized by François Wang in Marseille to harmonize the practice of the iMAX methodology between the 4 centers. The different parameters were recorded on three stimulation sites, using a bipolar surface stimulator (the type of stimulator was not imposed). The choice of stimulation points was based on the following criteria: sites where the nerves are superficial, proximal and distal, in the upper and lower limbs, with a maximum protocol time of 20 minutes allowing 3 sites. The exact stimulation sites were determined where the maximal CMAP could be obtained at minimal stimulus intensity as follow: around 2 cm proximal from the distal wrist crease for median nerve stimulation; above the medial epicondyle for ulnar nerve stimulation; at the lateral edge of the popliteal fossa for fibular nerve stimulation. Pregelled dis-

posable surface electrodes were used for recording (the type of electrode was not imposed). CMAPs were recorded from the *abductor pollicis brevis* muscle for median nerve stimulation (G1 at half distance from the midpoint of the distal wrist crease to the first metacarpophalangeal joint), the *adductor digiti minimi* muscle for ulnar nerve stimulation (G1 at the midpoint of the hypothenar eminence) and the *tibialis anterior* muscle for fibular nerve stimulation (G1 at proximal quarter of the distance from the tibial anterior tuberosity to medial malleolus). The reference electrodes (G2) were placed over the proximal phalanx of the thumb and the fifth finger for the median nerve and ulnar nerve respectively, and on the medial malleolus for the fibular nerve. The ground electrode was placed over the ventral part of the forearm or over the leg (hairless skin). We used the longest stimulus duration of 1 ms in order to minimize technical limitation in the case of a severe motor axon hypoexcitability. The bandpass filter setting was set from 2 to 5000 Hz. As voltage is the product of current by resistance (Ohm's law), skin impedances under recording and ground electrodes were kept less than 20 kOhm. Skin temperature was maintained above 30 °C. In patients, as high current intensity was sometimes required, a two-channel (c1/c2) recording was used (c1 thenar/c2 hypothenar, c1 hypothenar/c2 thenar, c1 *tibialis anterior*/c2 *abductor hallucis*) to recognize and not accept the situations where motor responses were the result of nerve coactivation.

The minimal motor threshold was first measured. It was the minimal intensity stimulation with a 1 ms duration evoking a reproducible motor response of at least 0.1 mV peak to peak amplitude. Then, the stimulus intensity was gradually increased, with increments of 1 mA, until a maximal CMAP amplitude (measured from baseline to the first negative peak) was obtained. The stimulation intensity at this stage was called iUP. A stimulation increased by 50% of this iUP was applied in order to ensure a maximum CMAP amplitude, with a precision of 0.1 mV (the iUP procedure had to be restarted if the 50% intensity increased induced an amplitude gain of the motor response). The stimulus intensity was then reduced progressively with decrements of 0.1 mA until a decline of the motor response was obtained. In practice, when the amplitude of CMAP decreased, at least a second stimulus was delivered at the same stimulus intensity to check whether or not there was a true decrease in CMAP (possibly related to a movement artifact). If the CMAP amplitude again reached the amplitude corresponding to iUP, a new decrement of 0.1 mA was tested and so on. Then, the stimulus intensity was increased anew with increments of 0.1 mA until reaching the maximal response evoked earlier. The stimulus intensity at this stage of the procedure was the iMAX, the lowest and most precise intensity allowing a maximal CMAP. If nerve coactivation was suspected, current intensity was not further increased and the iUP and iMAX were considered equal.

The recorded data during the procedure were minimal threshold, iUP and iMAX for the three stimulation sites.

2.4. Statistical analyses

Collected data included age, sex, height, weight, calculated body-mass index (BMI), and excitability parameters (threshold, iUP and iMAX).

Statistical analysis was performed by using SAS software (SAS University Edition, Cary, NC).

Descriptive statistics were expressed as quartiles (median, Q1 and Q3).

Given the variance heterogeneity and small size of samples, and due to the fact that some variables (for instance iUP and iMAX) were not distributed normally, only parametric tests were used: Wilcoxon signed-rank test for test–retest comparisons and gender influence study, Kruskal-Wallis for comparisons of more than 2 groups (groups being compared two by two by the Dwass, Steel, Critchlow-Fligner method if the overall model was relevant), Spearman rank correlation coefficient between test–retest data. The relation between excitability and clinical data (i.e., age, sex, height, weight and BMI) was studied by stepwise multiple regression analysis.

A two-sided p value < 0.05 was considered significant.

The upper limit of normal (ULN) for excitability parameters was established in the control group by the percentile method (P95).

3. Results

Overall, 28 healthy volunteers participated in the study (8 in Liège, 6 in Fraiture, 8 in Marseille and 6 in Nice) and 32 patients with peripheral neuropathies were recruited (13 CIDP, 8 GBS, and 7 CMT1A and 4 axonal neuropathy). None of our healthy controls reported symptoms or had neurological abnormalities on careful clinical examination suggestive of a peripheral nerve disease such as metabolic disorder, renal failure and toxic or entrapment neuropathies. All the patients with CMT1A presented a PMP22 duplication. The patients with CIDP had to fulfill European Federation of Neurological Societies/Peripheral Nerve Society criteria (Van den Bergh et al., 2010). The patients with GBS fulfilled either Asbury and Cornblath (1990) criteria (Marseille) or Rajabally et al. (2015) criteria (other centers). In the axonal neuropathy group, 2 patients were affected by transthyretin-related familial amyloid polyneuropathy, 1 patient had 9-year-old sequelae of AMAN and the last one had a vasculitis restricted to the peripheral nervous system.

3.1. Healthy volunteers

14 women and 14 men aged from 17 to 77 years old participated in the study. There were some significant differences related to gender. Men were taller and heavier than women. Regarding excitability parameters, only the iMAX of the fibular nerve at the knee was lower in men than in women.

3.1.1. Comparison of the four centers

As shown in tables 1 and 2, the value of the Kruskal-Wallis H statistic, with p > 0.05, led us to tolerate, in the overall population, the hypothesis of the equality of medians between the four centers for both clinical (age, height, weight and body mass index) and excitability data (threshold, iUP and iMAX) (Table 1).

3.1.2. Standards

As there was no significant difference between the four centers for each nerve and each parameter, we were able to establish standards, pooling the four centers (n = 28) (Table 2). ULN was defined as the 95th percentile (P95).

3.1.3. Regression analysis between excitability data and healthy volunteer's characteristics

Stepwise multiple regression analysis indicated that among age, height, weight and BMI, age only was an explicative variable for several dependant variables: minimal threshold (median and ulnar nerves), iUP (median and fibular nerves), iMAX (median nerve) (Table 3).

3.1.4. Inter-center and intra-center variability

For each individual center and all the centers pooled, there were no significant differences between the first (test) and the second test (retest) regarding minimal threshold, iMAX and iUP for each nerve using Wilcoxon test for paired sample (p > 0.05).

The test–retest reliability assesses the variability related to the technique and not the inter-subject or inter-nerve variability, thus

Table 1
clinical characteristics and excitability parameters of healthy volunteers (median values).

Nerves	Parameters	Marseille (n = 8)	Liège (n = 8)	Fraiture (n = 6)	Nice (n = 6)	Kruskal-Wallis
	Age (year)	34.5	38.5	54.0	48.5	p > 0.05
	Height (cm)	168.5	175.5	172.0	169.5	p > 0.05
	Weight (kg)	63	65.5	80.5	66.0	p > 0.05
	Body mass index	22.5	22.5	26.0	23.5	p > 0.05
Median	Minimal threshold (mA)	1.6	1.6	1.5	1.8	p > 0.05
	iUP (mA)	4.0	4.0	4.0	4.5	p > 0.05
	iMAX (mA)	3.6	3.9	3.8	3.6	p > 0.05
Ulnar	Minimal threshold (mA)	1.7	1.2	1.5	1.3	p > 0.05
	iUP (mA)	6.0	5.5	5.5	6.0	p > 0.05
	iMAX (mA)	5.8	4.8	5.1	4.7	p > 0.05
Fibular	Minimal threshold (mA)	1.5	1.7	2.1	1.5	p > 0.05
	iUP (mA)	5.0	5.5	7.0	5.0	p > 0.05
	iMAX (mA)	4.7	5.1	6.7	4.5	p > 0.05

Table 2
excitability parameters of healthy volunteers (the 4 centers grouped together; n = 28).

Nerve	Excitability Parameters (mA)	Median	Q1	Q3	P95
Median	Minimal threshold	1.6	1.4	1.9	2.3
	iUP	4.0	3.5	5.0	6.0
	iMAX	3.8	3.0	4.8	5.8
Ulnar	Minimal threshold	1.3	1.0	1.8	2.7
	iUP	6.0	4.5	6.5	7.0
	iMAX	5.1	3.9	5.8	6.8
Fibular	Minimal threshold	1.6	1.2	1.9	2.6
	iUP	5.5	5.0	7.0	8.0
	iMAX	5.0	4.1	6.3	7.5

Q: quartile, P95: the 95th percentile

Table 3
stepwise multiple regression analysis in healthy volunteers (n = 28).

Nerve	Dependent variables	Intercept	Regression coefficient			
			Age	Height	Weight	BMI
Median	Minimal threshold	1.0315 (p < 0.0001)	0,0128 (p = 0.0070)	p > 0.05	p > 0.05	p > 0.05
	iUP	2.4368 (p = 0.0001)	0.0439 (p = 0.0005)	p > 0.05	p > 0.05	p > 0.05
	iMAX	2.0345 (p < 0.0001)	0.0345 (p = 0.0016)	p > 0.05	p > 0.05	p > 0.05
Ulnar	Minimal threshold	0.7367 (p = 0.0250)	0.0166 (p = 0.0151)	p > 0.05	p > 0.05	p > 0.05
	iUP	no relevant model	p > 0.05	p > 0.05	p > 0.05	p > 0.05
	iMAX	no relevant model	p > 0.05	p > 0.05	p > 0.05	p > 0.05
Fibular	Minimal threshold	no relevant model	p > 0.05	p > 0.05	p > 0.05	p > 0.05
	iUP	4.0521 (p = 0.0002)	0.0398 (p = 0.0474)	p > 0.05	p > 0.05	p > 0.05
	iMAX	no relevant model	p > 0.05	p > 0.05	p > 0.05	p > 0.05

Spearman's correlation analysis was conducted pooling the four centers and the three nerves (n = 84). Correlation coefficients (R) between the test and retest data were statistically significant: 0.55 (p < 0.0001) for minimal threshold, 0.51 (p < 0.0001) for iUP and 0.55 (p < 0.0001) for iMAX (Fig. 1).

3.2. Patients with peripheral neuropathy

The characteristics of patients with peripheral neuropathy are depicted in Table 4. Comparing the five groups (axonal neuropathy, CMT, CIDP, GBS and healthy volunteers), there was a significant difference regarding age only. Groups being compared two by two by the Dwass, Steel, Critchlow-Fligner (DSCF) method indicated that the CMT1a group was younger than the GBS and the CIDP groups. Moreover, there was no significant difference in age between the group of healthy subjects and the groups of patients with neuropathy.

Table 5 summarizes excitability data obtained in the five study groups. The overall models tested were relevant (p < 0.0001). The Kruskal-Wallis analysis revealed that excitability parameters (iMAX, iUP and threshold) were significantly different between the five groups for the three nerves studied. Minimal threshold and iMAX are illustrated for each nerve in Fig. 2. When each group was compared to each other (DSCF method) strong differences between healthy volunteers and patients with CMT1a and between healthy volunteers and patients with CIDP were identified regarding all parameters and for the three nerves. Table 6 summarizes all significant differences between groups. In GBS patients, there were no significant differences between data recorded before day 14 from onset of symptoms and after the day 28. Table 7 summarizes the percentage of abnormalities by comparing each individual data with ULN. The last column evaluates the percentage of systematic abnormalities for the three studied parameters at the three stimulation sites (patients with 9 abnormal values).

4. Discussion

In current neurophysiological practice, by measuring CMAP amplitudes, distal motor latencies, conduction velocities, conduction blocks, or F-wave latencies, peripheral neuropathies are fairly well characterized. Nevertheless, some EDX results are sometimes confusing. It is not uncommon that a conduction slowing results

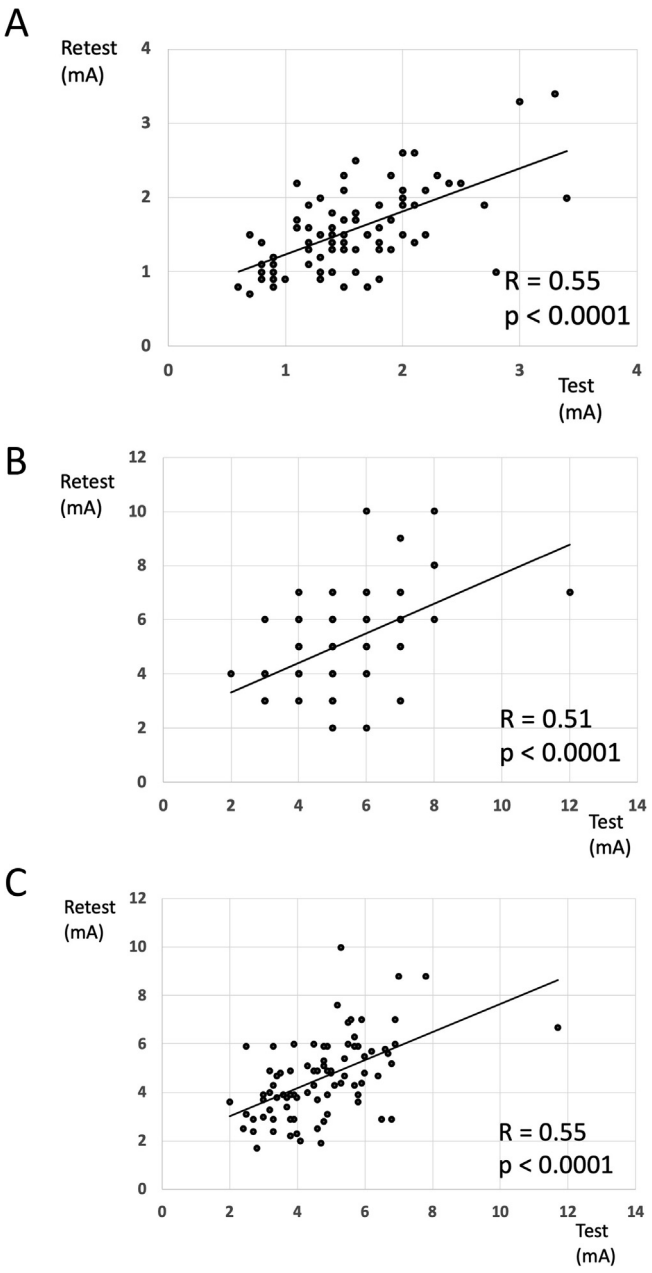

Fig. 1. Spearman correlation analysis between test and retest in the same population for **(A)** minimal threshold, **(B)** iUP i.e. the lowest stimulus intensity allowing a maximal response by 1 mA increments (30 visible dots and 54 superimposed dots) and **(C)** iMAX. The three nerves and the four centers are pooled (n = 84).

from a significant loss of fast conducting axons or that a low CMAP amplitude is due to a temporal dispersion. Moreover, a part of the EDX information is systematically lost, the amount of current that is required to obtain the maximal CMAP. Yet everyone has experienced that a significantly higher stimulus intensity is required to evoke a supramaximal motor response in a patient with demyelinating neuropathy. This is due to a motor axon hypoexcitability. Hypoexcitability mainly results from changes in the properties of the axonal membrane and the ion channels expressed on these axons. The study of the excitability of motor axons offers a better understanding of the pathophysiological mechanisms underlying neurological diseases. Moreover, nerve excitability testing might be a diagnostic tool and an interesting measure of response to therapy (Boërio et al, 2010). In their consensus guidelines, Kiernan et al. (2020) state that "Current axonal excitability protocols utilise threshold tracking as a preferred technique". We agree with this, nevertheless, a fast technique that does not require dedicated devices that would be carried out at the same time as the nerve conduction studies could prove to be very useful for clinical purposes, this is the role that the iMAX proposes to fulfill.

It should also be noted that this study was conducted to establish the reliability of the iMAX method and show that it had its place in future multicenter studies. Groups of patients with neuropathy have been studied more to illustrate the feasibility and sensitivity of the method than with the aim of drawing conclusions about the different axonal excitability disorders in these neuropathies. For this purpose, studies will soon be carried out specifically in each type of neuropathy with a larger number of patients and taking into account in particular the duration of the disease and the timing of the examination in relation to the treatments from which the patients benefit and which could significantly influence axonal excitability.

In 2017, Milants et al. proposed the iMAX as a promising and sensitive parameter to assess peripheral motor axonal hypoexcitability in routine practice. A similar methodology was already used in patients with chronic motor neuropathy (Takanori et al., 1996; Priori et al., 2002) and with motor neuron disease (Priori et al., 2002). The question of whether iMAX can offer clinicians anything new was already discussed (Burke and Kiernan, 2018; Wang, 2018). Indeed, excitability properties of human peripheral nerves can be assessed by various neurophysiological methods (Brismar, 1985; Bostock et al., 1998; Kiernan et al., 2020), but to date, iMAX might be the only one allowing everyone, in daily practice and whatever the EDX machine, to answer very quickly the question of whether or not there is a motor axonal hypoexcitability and what its extent is. In addition, if it is correct that by considering only iMAX, a valuable information related to the stimulus–response curve is lost, particularly the curve slope (Burke and Kiernan, 2018), the delta between the values of iMAX and minimal threshold contains information similar to the curve slope (Fig. 3).

The first part of the study in healthy volunteers aimed to confirm the reliability of the iMAX procedure. The multicenter study makes it possible to test the within and between-center variability of this technique. There were no significant inter-center differences for each nerve tested regarding iMAX, iUP and minimal threshold (Table 1, Kruskal-Wallis test). Intra-center reliability of excitability parameters over time was good too. There was no significant difference between the test and retest for the four centers

Table 4
clinical characteristics of healthy volunteers (n = 28) and patients with peripheral neuropathies (n = 32) (median values).

Parameters	Healthy (n = 28)	Axonal (n = 4)	GBS (n = 8)	CIDP (n = 13)	CMT1a (n = 7)	Kruskal-Wallis
Age (year)	47.5	62.5	52.5*	63.0*	35.0*	p = 0.0151
Height (cm)	172.0	177.0	166.5	178.0	168.0	p > 0.05
Weight (kg)	66.0	77.8	84.0	80.0	57.0	p > 0.05
BMI	23.5	25.8	26.0	25.0	20.0	p > 0.05

BMI: body mass index, GBS: Guillain-Barré syndrome, CIDP: chronic inflammatory demyelinating polyneuropathy, CMT: Charcot-Marie-Tooth
*CMT1a group was younger than GBS and CIDP groups (Dwass, Steel, Critchlow-Fligner method)

Table 5
median data of excitability parameters in healthy volunteers (n = 28) and in patients with peripheral neuropathies (n = 32).

	Excitability parameters (mA)	Median nerve	Ulnar nerve	Fibular nerve
Healthy volunteers (n = 28)	Minimal threshold	1.6	1.3	1.6
	iUP	4.0	6.0	5.5
	iMAX	3.8	5.1	5.0
Axonal neuropathies(n = 4)	Minimal threshold	2.0	1.5	1.6
	iUP	8.5	6.0	8.0
	iMAX	7.9	4.7	7.6
GBS (n = 8)	Minimal threshold	2.3	2.2	1.9
	iUP	11.9	9.0	7.0
	iMAX	10.9	8.3	6.5
CIDP (n = 13)	Minimal threshold	3.2	3.2	2.3
	iUP	14.0	14.0	9.5
	iMAX	13.3	12.8	8.7
CMT1a (n = 7)	Minimal threshold	6.6	6.2	6.4
	iUP	26.0	26.0	29.0
	iMAX	26.0	26.0	29.0

GBS: Guillain-Barré syndrome, CIDP: chronic inflammatory demyelinating polyneuropathy, CMT: Charcot-Marie-Tooth

and the three nerves (Wilcoxon test for paired samples). Correlation coefficients (R) between test and retest were moderate (slightly greater than 0.5), but these relationships were highly significant ($p < 0.0001$) (Fig. 1, Spearman correlation). We postulate a better test–retest reliability in a single-center study (only one investigator). From the perspective of an evaluation of response to a therapy, a composite score could provide diagnostic value.

Stepwise multiple regression was conducted to emphasize if our measurements had to be adapted depending on subject characteristics (age, height, weight, BMI). Based on our findings, only age should be taken into account when evaluating motor axon excitability by iMAX procedure (Table 3: intercepts and regression coefficients). In the present work, weight and BMI do not significantly influence the parameters of excitability. However, edema or obesity has not been specifically studied. As the ability of an electrical stimulus to excite motor axons partly depends on impedance of intervening tissues, it can be postulated that significant edema or obesity impact the results by increasing the iMAX parameters. ULN were established using percentile 95 for the three nerves (Table 2). These results confirm that the iMAX methodology is reliable and may be used in a multicentric manner. Moreover, excitability data may be obtained from any site of the peripheral nervous system. In the present study, three distinct sites (median nerve at wrist, ulnar nerve at elbow, fibular nerve at knee) were investigated. As already said by Milants et al. (2017), iMAX procedure is also a fast technique which may be achieved in around 5 minutes per stimulation site.

Gender influence study (Wilcoxon signed-rank test) on excitability parameters only revealed lower iMAX values at the knee in men than in women. An increased capacitance around the nerve fibers, potentially linked to fatty tissue at the knee in females, is postulated.

The second part of the study compared minimal threshold, iMAX and iUP between healthy volunteers, patients with axonal neuropathies and patients with demyelinating neuropathies (CMT1a, CIDP, GBS). This part remains preliminary. Larger studies should be conducted for more definitive conclusions. The main question was to know whether iMAX is sensitive enough to distinguish a healthy population from a population affected by peripheral neuropathy. Moreover, we hypothesized that iMAX might be helpful to discriminate axonal versus demyelinating polyneuropathies and possibly acquired demyelinating (segmental abnormalities of excitability) versus inherited demyelinating (diffuse abnormalities of excitability) polyneuropathies. Significant differences were found for the three parameters measured between healthy volunteers and patients with demyelinating neuropathies especially CMT1a and CIDP (Fig. 2, Table 6, DSCF method). In CMT1a patients, motor axon hypoexcitability was particularly pronounced and systematically concerned the three studied parameters (minimal threshold, iUP, iMAX) at the three stimulation sites which was not the case for CIDP patients (Table 7). In a very similar approach (stimulus intensity value required to obtain a supramaximal CMAP), although retrospective, Parker et al (2016) observed in median and ulnar studies that mean supramaximal intensities were significantly higher in patients with CMT and CIDP than normal controls. Many studies have also been conducted using Qtrac software (threshold tracking techniques) to study motor nerve excitability in demyelinating neuropathies. Two studies conducted in CMT1a (Nodera et al., 2004; Sung et al., 2004) identified an increase in stimulation threshold with "fanning-out" (spreading and more pronounced curvature) of threshold electrotonus and changes in the recovery cycle. These excitability abnormalities might be caused by exposure or spread of the potassium channels from under the myelin in a demyelinating pathology (Nodera et al., 2004). Two studies conducted in CIDP reported multiple abnormalities in excitability measurements for typical CIDP (Cappelen-Smith et al., 2001; Sung et al., 2004). Cappelen-Smith et al. (2001) demonstrated an alteration of stimulus–response curves (higher threshold, reduce slope) and higher rheobase in patients with CIDP than in healthy controls without any changes in threshold electrotonus. Though, Sung et al. (2004) demonstrated significant "fanning-out" of threshold electrotonus in CIDP. They state that threshold electrotonus can be used to detect demyelination at the tested sites and may provide information about the distribution patterns of demyelination in CIDP. In the present study, we only measured minimal threshold and iMAX in one site on each nerve. It would be interesting to study different stimulation sites in the same nerve (inching studies) in segmental demyelinating neuropathies as CIDP or even MMN.

Excitability disorders in GBS are more controversial. In our study, there were significant differences with healthy volunteers for minimal threshold (ulnar nerve), iMAX and iUP (median and ulnar nerves) (Fig. 2, Table 6). Pyun et al. (2017) compared axonal and demyelinating forms of GBS. In their study, there were no excitability disorders in demyelinating forms while there was high refractoriness (the increase in threshold during the relative refractory period) in axonal forms, without any other excitability disorder. It would suggest that antibodies against axonal membrane impairs nodal structures such as sodium channels. Nevertheless, in the present study patients with both axonal and demyelinating GBS forms presented abnormalities of motor axon excitability (Table 7). A recent study using CMAP scans (EMG method in which the build-up of the CMAP is visualized) confirms some of our results by demonstrating more severe hypoexcitability in AIDP

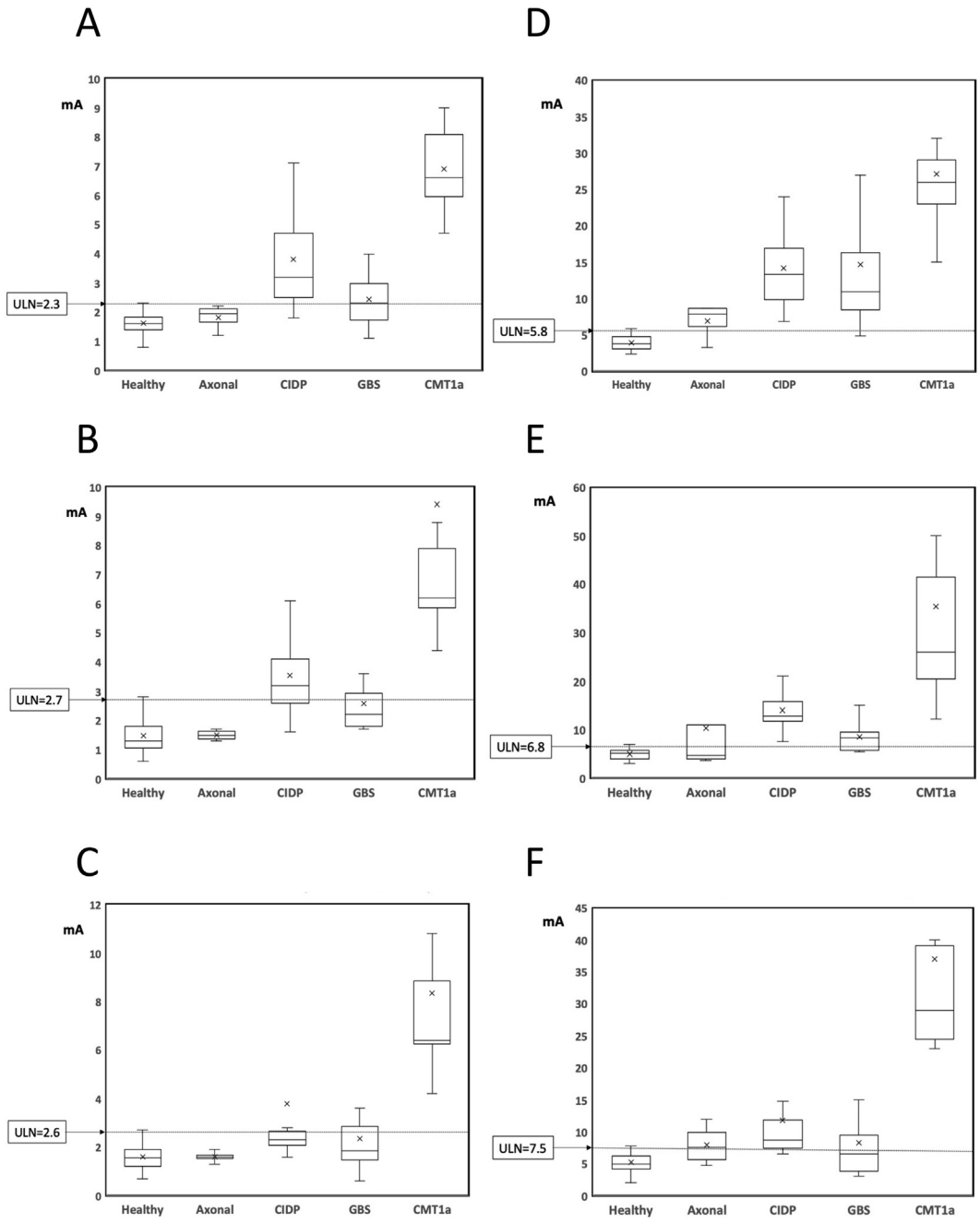

Fig. 2. Box plot comparing minimal threshold **(A, B, C)** and iMAX **(D, E, F)** recorded from median **(A, D)**, ulnar **(B, E)** and fibular **(C, F)** nerves among the five groups: healthy volunteers (n = 28), axonal neuropathies (n = 4), chronic inflammatory demyelinating polyneuropathy (CIDP) (n = 13), Guillain-Barré syndrome (GBS) (n = 8), Charcot-Marie-Tooth (CMT1a) (n = 7); ULN = upper limit of normal (P95).

Table 6
p value less than 0.05 for group comparisons 2 by 2 by Dwass, Steel, Critchlow-Fligner method.

	Minimal threshold			iUP			iMAX		
	Median nerve	Ulnar nerve	Fibular nerve	Median nerve	Ulnar nerve	Fibular nerve	Median nerve	Ulnar nerve	Fibular nerve
CMT1a vs Healthy	0.0005	0.0005	0.0005	0.0004	0.0004	0.0004	0.0005	0.0005	0.0005
CMT1a vs CIDP		0.0088	0.0326	0.0115	0.0376	0.0124	0.0169	0.0490	0.0152
CMT1a vs GBS	0.0105	0.0151	0.0152		0.0210	0.0100		0.0153	0.0104
Healthy vs CIDP	<0.0001	<0.0001	0.0026	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	0.0001
Healthy vs GBS		0.0193		0.0007	0.0211		0.0005	0.0113	

GBS: Guillain-Barré syndrome, CIDP: chronic inflammatory demyelinating polyneuropathy, CMT: Charcot-Marie-Tooth
Missing values mean the p-value was > 0.05

Table 7
percentage of abnormalities in patients with peripheral neuropathies for the three stimulation sites, established by comparison of individual data to upper limits of normal.

	Parameters	Median nerve	Ulnar nerve	Fibular nerve	Three abnormal parameters at the 3 sites
Axonal neuropathies (n = 4)	Minimal threshold (%)	0	0	0	0
	iUP (%)	75	25	50	
	iMAX (%)	75	25	50	
GBS axonal form (n = 3)	Minimal threshold (%)	67	67	33	33
	iUP (%)	100	67	33	
	iMAX (%)	100	67	33	
GBS demyelinating form (n = 5)	Minimal threshold (%)	40	20	20	0
	iUP (%)	80	60	20	
	iMAX (%)	80	60	20	
CIDP (n = 13)	Minimal threshold (%)	77	77	25	0
	iUP (%)	100	92	50	
	iMAX (%)	100	91	50	
CMT1a (n = 7)	Minimal threshold (%)	100	100	100	100
	iUP (%)	100	100	100	
	iMAX (%)	100	100	100	

GBS: Guillain-Barré syndrome, CIDP: chronic inflammatory demyelinating polyneuropathy, CMT: Charcot-Marie-Tooth

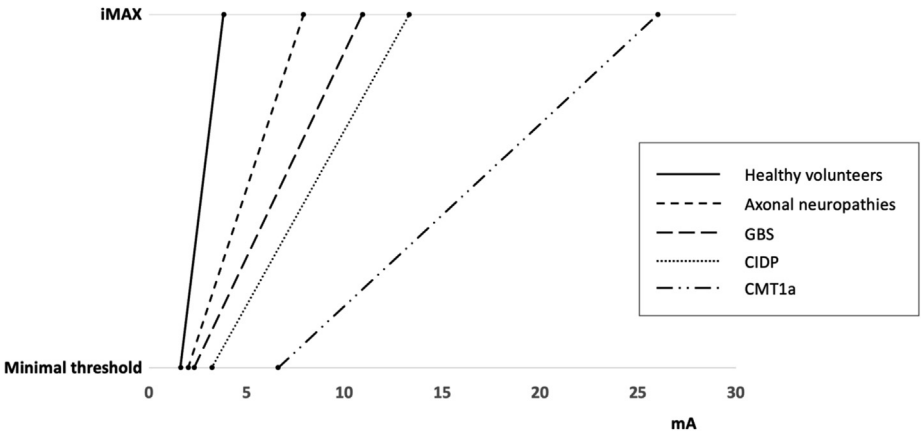

Fig. 3. Median values for minimal threshold and iMAX recorded from the median nerve among the five groups: healthy volunteers (n = 28), axonal neuropathies (n = 4), chronic inflammatory demyelinating polyneuropathy (CIDP) (n = 13), Guillain-Barré syndrome (GBS) (n = 8), Charcot-Marie-Tooth (CMT1a) (n = 7).

patients than in AMAN patients. The most pronounced differences between groups were found, particularly at S100, the intensity of the stimulus activating all motor units (Drenthen et al, 2021). The absence of significant difference between iMAX data recorded before day 14 from onset of symptoms and after day 28 may be due to the small sample of GBS (n = 8 and only 5 patients with complete data after day 28) and the fact that by the second evaluation some patients had got better and others had got worse.

There was no significant difference between patients with axonal neuropathies and healthy volunteers for each parameter (minimal threshold, iMAX, iUP). However, we could observe a slight upward trend of iMAX in axonal neuropathies (Fig. 2). Moreover, Table 7 shows clearly that abnormalities of motor axon excitability may be found in patients with axonal neuropathies. Our study included four axonal neuropathies, two amyloidosis, one vasculitis with sequalae and one AMAN with sequalae. A number of studies have already been conducted to study motor axons excitability disorders in axonal neuropathies. Most of these studies have been done in diabetic neuropathies. In this pathology, excitability findings show an increase in relative refractory period, a decrease in superexcitability and subexcitability, and a "fanning- in" (flattening and less pronounced curvature) appearance of threshold electrotonus (Kuwabara et al., 2002; Misawa et al., 2005; Bae et al., 2011; Sung et al., 2012). Diabetes would reflect altered sodium channel function, an alteration of Na^+/K^+ pump function, membrane depolarization, and increase ischemic resistance even in the subclinical early stage of diabetes (Bae et al., 2011). The same excitability disorders are found in patients with chronic kidney disease (Kiernan et al., 2002; Krishnan et al., 2005, 2006). These findings might come from axonal depolarization, most likely driven by hyperkalaemia prior to dialysis. Excitability disorders in axonal neuropathies would depend on the pathophysiology of these neuropathies. Axonal neuropathies with distal dying back would present fewer excitability disorders (loss of large fibers that have low thresholds for excitation) than pathologies which disturb the functioning of ionic channels or pumps as in ischemic neuropathies. Further investigation should be conducted.

In the present study, iUP and iMAX seem redundant. The two parameters evolve in parallel and the results are almost identical both in volunteers and in patients with peripheral neuropathy. iUP is obtained faster, but iMAX is more precise. Here also, further investigation is needed to decide which one is preferable. Conversely, the minimal threshold and the iMAX are not equivalent or interchangeable. The data in Table 7 indicate that in patients with neuropathy, iMAX values are more often greater than ULN than are minimal threshold values. The minimal threshold studies only the most excitable axons whose threshold can remain within the normal limits, in particular in the event of axonal neuropathy or inflammatory neuropathy. iMAX integrates the excitability of all motor axons including the least excitable axons.

This study confirms that the iMAX procedure can highlight motor axon excitability disorders and could be useful to distinguish a healthy population from a population affected by a demyelinating neuropathy. Their measurement does not require

any specific software and the technique used is fast, non-invasive and measurable at any stimulation point over any nervous trunk. The iMAX procedure seems reliable with only a small, not statistically significant, variation between different centers and between two tests in the same subjects. A study should be conducted with a larger sample of healthy volunteers and of patients with peripheral neuropathies. It should be of interest to assess the relationships between axonal excitability parameters, derived from the iMAX procedure, and classical EDX parameters such as motor conduction velocity, distal and F latencies, and CMAP size. We plan also to assess the respective contributions of iMAX and techniques already existing measuring motor axon excitability, especially with Qtrac device, in the same populations.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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