Neurophysiologie Clinique/Clinical Neurophysiology (2008) **38**, 31—38

Disponible en ligne sur www.sciencedirect.com

ScienceDirect

journal homepage: http://france.elsevier.com/direct/neucli

ORIGINAL ARTICLE/ARTICLE ORIGINAL

Stimulus—response curve of human motor nerves: Multicenter assessment of various indexes

Évaluation multicentrique de différents indices de la courbe stimulus—réponse des nerfs moteurs chez l'homme

D. Boërio[a,b], J.-Y. Hogrel[b], J.-P. Lefaucheur[a,*], F.C. Wang[c], A. Verschueren[d], J. Pouget[d], E. Carrera[e], T. Kuntzer[e,*]

[a] *Service de physiologie—explorations fonctionnelles, hôpital Henri-Mondor, AP—HP, 94010 Créteil, France*
[b] *Institut de myologie, groupe hospitalier Pitié-Salpêtrière, 75013 Paris, France*
[c] *Département de neurophysiologie, CHU Sart-Tilman, 4000 Liège, Belgium*
[d] *Département de neurologie et maladies neuromusculaires, CHU La-Timone, 13005 Marseille, France*
[e] *Unité nerf—muscle, service de neurologie, centre hospitalier universitaire Vaudois, 1011 Lausanne, Switzerland*

Received 18 March 2007; accepted 9 September 2007
Available online 11 October 2007

KEYWORDS
Intensity threshold;
Membrane properties;
Nerve excitability;
Normative data;
Stimulus duration;
Stimulus intensity;
Stimulus—response curve

Summary The value of various indexes to characterize the stimulus—response curve of human motor nerves was assessed in 40 healthy subjects recruited from four European centers of investigation (Créteil, Lausanne, Liège, Marseille). Stimulus—response curves were established by stimulating the right median and ulnar motor nerves at the wrist, with stimulus durations of 0.05 and 0.5 ms. The following parameters were studied: the threshold intensity of stimulation to obtain 10% (I_{10}), 50% (I_{50}), and 90% (I_{90}) of the maximal compound muscle action potential, the ratios I_{10}/I_{50}, I_{90}/I_{50}, $(I_{90}-I_{10})/I_{10}$, $(I_{90}-I_{50})/I_{50}$, and $(I_{50}-I_{10})/I_{10}$, and the slopes of the stimulus—response curves with or without normalization to I_{50}. For each parameter, within-center variability and reproducibility (in a test—retest study) were assessed and between-center comparisons were made. For most of the parameters, the results varied significantly within and between the centers. Within the centers, only the ratios I_{10}/I_{50} and I_{90}/I_{50} were found constant and reproducible. Between the centers, the absolute intensity thresholds (I_{10}, I_{50}, I_{90}) and the ratio I_{90}/I_{50} did not show significant differences at stimulus duration of 0.5 ms, whatever the stimulated nerve. The reduced variability and good reproducibility of the ratios I_{10}/I_{50} and I_{90}/I_{50} open perspectives in neurophysiological practice for the use of these indexes of the stimulus—response curve, a rapid and noninvasive test.

* Corresponding authors.
E-mail addresses: jean-pascal.lefaucheur@hmn.aphp.fr (J.-P. Lefaucheur), thierry.kuntzer@chuv.ch (T. Kuntzer).

0987-7053/$ — see front matter © 2007 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.neucli.2007.09.007

MOTS CLÉS
Courbe stimulus—réponse ;
Données normatives ;
Durée de stimulation ;
Excitabilité nerveuse ;
Intensité de stimulation ;
Propriétés membranaires ;
Seuil d'intensité

Résumé La valeur de différents indices caractérisant la courbe stimulus—réponse des nerfs moteurs a été étudiée dans une population de 40 sujets sains recrutés dans quatre centres européens (Créteil, Lausanne, Liège, Marseille). Les courbes stimulus—réponse des nerfs médian et cubital ont été obtenues en délivrant des stimulations au poignet droit d'une durée de 0,05 et 0,5 millisecondes. Nous avons étudié les seuils d'intensité de stimulation permettant d'obtenir 10 % (I_{10}), 50 % (I_{50}) et 90 % (I_{90}) de la valeur maximale du potentiel d'action moteur global, les rapports I_{10}/I_{50}, I_{90}/I_{50}, $(I_{90}-I_{10})/I_{10}$, $(I_{90}-I_{50})/I_{50}$ et $(I_{50}-I_{10})/I_{10}$, ainsi que les pentes des courbes stimulus—réponse avec ou sans normalisation par I_{50}. Pour chaque paramètre, la variabilité et la reproductibilité (lors d'une étude test—retest) ont été évaluées dans les différents centres et des comparaisons ont été réalisées entre les centres. Pour la plupart des paramètres, les résultats variaient significativement aussi bien dans chaque centre qu'entre les centres. Seuls les rapports I_{10}/I_{50} et I_{90}/I_{50} furent trouvés constants et reproductibles dans chacun des centres. Par ailleurs, les seuils d'intensité absolus (I_{10}, I_{50}, I_{90}) et le rapport I_{90}/I_{50} ne montrèrent pas de différences significatives entre les centres pour une durée de stimulation de 0,5 millisecondes, quel que soit le nerf stimulé. La faible variabilité et la bonne reproductibilité des rapports I_{10}/I_{50} et I_{90}/I_{50} ouvrent des perspectives pour l'application en pratique neurophysiologique de ces indices qui caractérisent la courbe stimulus—réponse, un test rapide et non invasif.

Introduction

Excitability properties of human peripheral nerves can be assessed by various neurophysiological methods [3,5]. One of these methods aims at measuring the stimulus intensities that are required for eliciting compound muscle action potentials (CMAPs) of given amplitudes, corresponding to various percentages of the maximal CMAP area or amplitude (M_{max}). The resulting ''stimulus—response'' curve is classically established for stimulus durations of 0.2 and 1 ms. This method, first developed by Brismar [2] to study metabolic neuropathies, was then applied by Meulstee et al. [10] in patients with demyelinating neuropathies. Various parameters have been proposed to characterize the stimulus—response curve, like the threshold intensities to obtain 10% (I_{10}), 50% (I_{50}) and 90% (I_{90}) of M_{max}, the ratio $(I_{90}-I_{10})/I_{10}$ [2] and the slope of the curve. Later, Kiernan et al. [5] proposed to analyze the curves by normalizing threshold intensities as percentages of I_{50}.

Altered intensity thresholds reflect various pathophysiological processes, that is, modification of nodal properties, increase in nerve capacitance (owing to the presence of endoneural edema or demyelination), or selective loss of the largest nerve fibers. Abnormalities of the stimulus—response curve have been reported in patients with diabetic or uremic neuropathy [2], acute or chronic inflammatory demyelinating neuropathy [4,9,10], motor multifocal neuropathy [7], or amyotrophic lateral sclerosis [12]. Nevertheless, the methodology of this test has been rarely questioned.

The present study was conducted in four European laboratories of clinical neurophysiology (Créteil, Lausanne, Liège, Marseille) and included 10 healthy subjects per center. The stimulus—response curves were established for the median and ulnar motor nerves in all subjects at two stimulus durations (0.05 and 0.5 ms). Our goal was to appraise the respective value of various indexes (previously used or original ones) that characterize the stimulus—response curve of human motor nerves with a focus on within- and between-center variability and on test—retest reproducibility.

Methods

Subjects

Forty healthy volunteers participated to the study. Ten subjects were recruited from each of the following centers: Créteil, Lausanne, Liège, and Marseille. They were 16 women aged from 20 to 50 years (mean $\pm$ S.D. = 34.0 $\pm$ 10.1) and 24 men aged from 22 to 49 years (mean $\pm$ S.D. = 34.1 $\pm$ 8.5). None of these subjects presented any clinical or electrophysiological sign of peripheral nerve disorder, either diffuse or focal, including entrapment neuropathy (carpal tunnel syndrome or ulnar nerve lesion at elbow). In addition, they did not present any risk factor for peripheral neuropathy, including diabetes, alcohol abuse or neurotoxic drug intake.

Investigation technique

Electrophysiological testing was performed with a Keypoint EMG machine (Medtronic Functional Diagnostics, Skovlunde, Denmark) in Créteil and a Viking IV EMG machine (Nicolet, Viasys Healthcare Inc., Conshohocken, PA, USA) in Lausanne, Liège and Marseille. Electrical stimuli were delivered at a frequency of 0.5 Hz. The cathode was placed over the median or ulnar nerve at the right wrist, approximately 1 cm proximal to the most distal crease, where the maximal CMAP could be obtained at minimal stimulus intensity. The anode was placed 4 cm more proximally at the dorsal aspect of the forearm. To ensure a maximal depolarization of the nerve fibers and to minimize the effects of phase cancellation, monopolar stimulation at a single distal site was preferred to bipolar stimulation and multiple stimulation sites. In response to such monopolar stimulation of the median or ulnar nerve, CMAPs were recorded from the abductor pollicis brevis (APB) or the adductor digiti minimi (ADM) muscle with a belly-tendon montage (the active electrode at the motor point and the reference at the proximal phalanx).

Table 1 Demographic characteristics (age, sex) and mean (S.D.) skin temperature and resistance at stimulation site.

			Temperature (°C)		Resistance (kΩ)	
	Age	Sex	Ulnar	Median	Ulnar	Median
Créteil	31.6 (5.1)	4F—6M	31.0 (1.5)	30.9 (1.5)	11.4 (2.1)	11.4 (2.3)
Lausanne	29.4 (9.9)	3F—7M	32.6 (0.6)	31.9 (0.7)	26.5 (15.2)	25.1 (8.0)
Liège	26.7 (7.8)	3F—7M	33.6 (1.0)	33.6 (1.2)	16.3 (3.1)	16.1 (3.2)
Marseille	38.6 (10.4)	6F—4M	31.6 (0.5)	31.6 (0.6)	39.1 (7.4)	38.7 (8.5)

Pregelled disposable surface electrodes (#9013S0241, Medtronic Functional Diagnostics) were used for stimulation and recording in Créteil and Marseille (contact area: 9×6 mm). In Lausanne and Liège, pregelled disposable surface electrodes (# 019-766300, Nicolet, Viasys Healthcare Inc.) were used for recording (contact area: 22×32 mm) but reusable cup electrodes (# 019-411800, Nicolet, Viasys Healthcare Inc.) were used for stimulation (contact area: 10 mm in diameter). Before each session, the skin was carefully cleaned at both stimulation and recording sites where resistances were measured and maintained under 50 kOhms throughout the session. Skin temperature was also controlled and maintained at 32 ± 3.5 °C near the stimulating site in all cases. Mean values of skin temperature and resistance at the stimulation sites are presented for each center in Table 1. The signal was filtered through 20-Hz high-pass filter and 10,000-Hz low-pass filter.

First, stimulus intensity was set at a supramaximal value to determine maximal CMAP area. Then, the intensity thresholds required to obtain from 10% to 90% of the maximal CMAP area were measured with 10% steps (I_{10} to I_{90}) and progressively increasing or decreasing stimulus intensity. We did not fix a minimal number of stimuli to determine intensity threshold at each 10% step of the maximal CMAP area. The whole CMAP area was measured from the onset of the negative peak to the return to the baseline following the positive peak. The Viking IV EMG machine users performed the test with the MUNE program. The procedure was applied for each nerve at two stimulus durations (0.05 and 0.5 ms). Reproducibility (or, more precisely, repeatability) was assessed by performing the test twice in the same subjects with the same examiner and more than two days apart between test and retest examinations. The reproducibility study was done in three investigation centers (Créteil, Lausanne, Liège) and not in Marseille.

Analysis methods

The stimulus—response curves were analyzed using a computer-assisted method based on a nonlinear regression curve fit model (Prism 4, Graph Pad Software, Inc., San Diego, USA). The curve equation was that of a sigmoid function with a variable slope as follows, on which a Richard coefficient with two additional constraints (*i.e.*, bottom to 0 and top to 1) was applied to better fit the experimental data:

$$y = b + \frac{t - b}{(1 + 10^{(E-x).h})^{s}}$$

$$x = E - \frac{\log 10\left[\left(\frac{y-b}{t-b}\right)^{-\frac{1}{s}} - 1\right]}{h}$$

(b, lower level; t, upper level; E, X-coordinate logarithm of inflexion point; h, Hill slope; s, symmetry parameter).

For each stimulated nerve (median, ulnar), at each stimulus duration (0.05, 0.5 ms), the analyzed parameters were the threshold intensities I_{10}, I_{50} and I_{90}, and the slope of the stimulus—response curve, which was estimated as follows:

$$\text{Slope} = \left[\frac{0.2}{(I60 - I40)}\right]$$

In addition, the following threshold ratios (''Brismar-type'' ratios), derived from that proposed by Brismar [2], were calculated:

$$\frac{[I90 - I10]}{I10};\ \frac{[I90 - I50]}{I50};\ \frac{[I50 - I10]}{I10}$$

Finally, as proposed by Kiernan et al. [5], we divided all intensity thresholds by I_{50}, thus, providing normalized stimulus—response curves with respect to I_{50}. From these calculations, three additional indexes were analyzed that were the ''normalized'' ratios I_{10}/I_{50} and I_{90}/I_{50}, and the slope of the normalized curves.

Regarding the test—retest procedure, a coefficient of reproducibility (CoR, in %) was determined for each parameter from the two measurements (T, test and R, retest) as [1,11]:

$$\text{CoR} = 100\sqrt{2}\frac{|T - R|}{(T + R)}.$$

Statistical analyses

Statistical analyses were performed with the InStat 3 software (Graph Pad Software, Inc., San Diego, USA). Since all data did not pass the normality test (Kolmogorov—Smirnov method), nonparametric tests were used in all cases. Within the centers, the coefficients of variation (CoVs, defined as the ratio of standard deviation to the mean) and the CoRs were calculated for each parameter to determine the least variable and the most reproducible parameters, respectively. The CoVs and the CoRs were compared among the centers on the whole sets of data or for each specific parameter using a one-way ANOVA (Kruskal—Wallis (KW) test).

In each center, the whole sets of data were also compared according to the type of stimulated nerve (median nerve versus ulnar nerve) using a Wilcoxon matched-pairs signed-ranks test.

The KW test was then applied to compare the stimulus–response curve data among the four centers regarding each of the 10 studied parameters (threshold intensities, ratios, slope estimates). Age, skin temperature and resistance at stimulation site were also compared among the centers using the KW test, while a Chi-squared test was used to assess gender influence. The correlations between skin temperature or resistance and the values provided by the stimulus–response curves were assessed with the Spearman correlation test.

For within-center analyses, the level of statistical significance was set at $p < 0.05$. For between-center comparisons, a Bonferroni correction for multiple comparisons was applied and the level of significance was set at $p < 0.005$ (10 parameters being studied).

Results

The test was completed in all the subjects within 20 min without any adverse event. The averaged stimulus–response curves without and with normalization to I_{50} are illustrated for each center in Figs. 1 and 2. Mean values of the various parameters of the stimulus–response curve (thresholds, ratios, and slope estimates) are presented for each center in Table 2. Within-center variability and reproducibility were assessed first, and then comparisons were made between the centers.

Variability and reproducibility

The CoVs of the various parameters of the stimulus–response curve are presented for each center in Table 3. A low CoV characterized a highly constant parameter within a center. From these results, it appeared that the ''normalized'' ratios I_{10}/I_{50} and I_{90}/I_{50} were clearly the least variable parameters (CoVs ranging from 0.01 to 0.24), compared to intensity thresholds (CoVs ranging from 0.16 to 0.62), slope estimates (CoVs ranging from 0.15 to 0.73), and ''Brismar-type'' ratios (CoVs ranging from 0.15 to 1.13). On the whole, the CoVs differed between the centers ($p = 0.0006$, KW test) and lower CoVs were observed in Créteil versus Lausanne ($p < 0.001$, Dunn's post-hoc test) and Liège ($p < 0.01$).

A reproducible parameter was characterized by a low CoR. The mean CoRs calculated in the test–retest study are presented in Table 3. It appeared that the ''normalized'' ratios I_{10}/I_{50} and I_{90}/I_{50} were clearly the most reproducible parameters (mean CoRs ranging from 2.09 to 8.26), compared to intensity thresholds (mean CoRs ranging from 13.58 to 28.88), slope estimates (mean CoRs ranging from 15.12 to 35.15), and ''Brismar-type'' ratios (mean CoRs ranging from 14.41 to 36.83). The CoRs did not differ between the

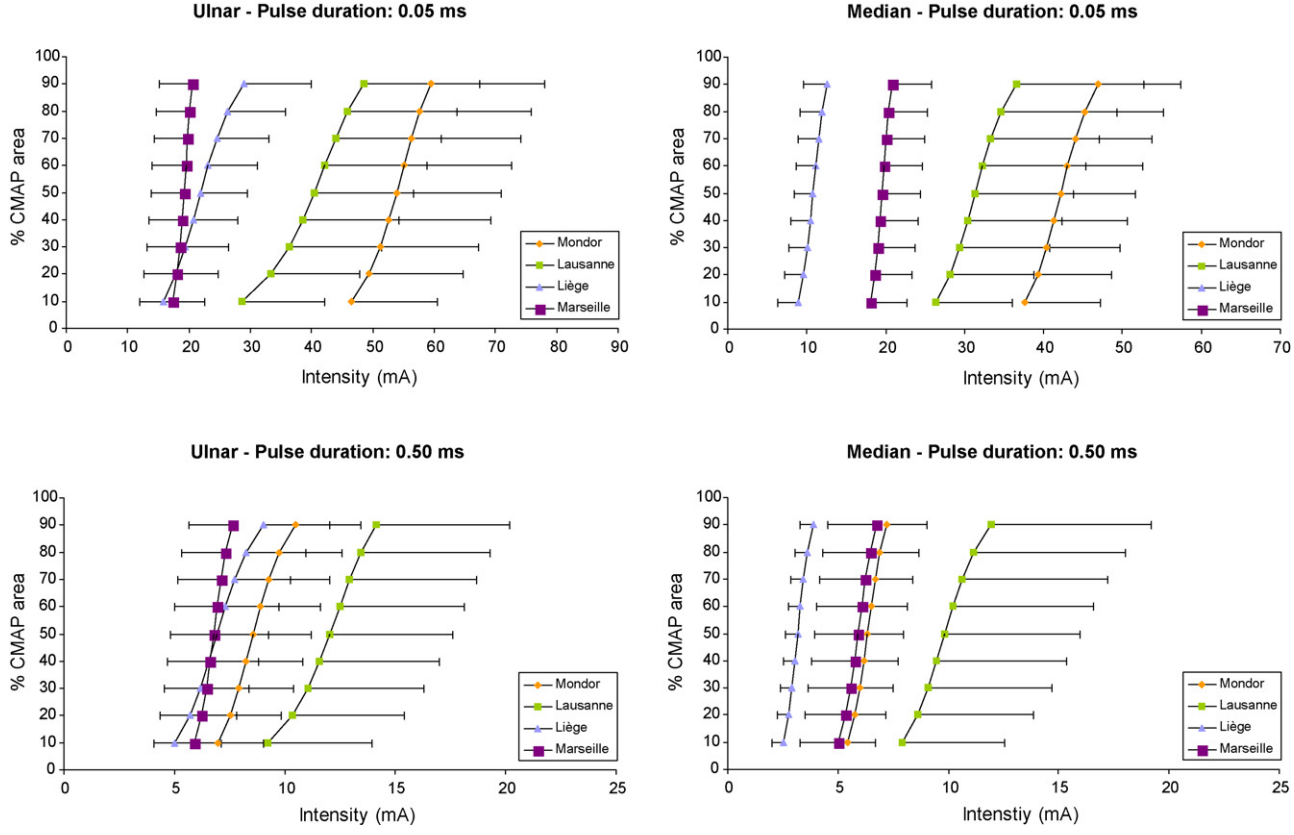

Figure 1 Averaged (S.D.) stimulus–response curves of the ulnar and median nerves stimulated at two different stimulus durations (0.05 and 0.5 ms) in the four centers of investigation.

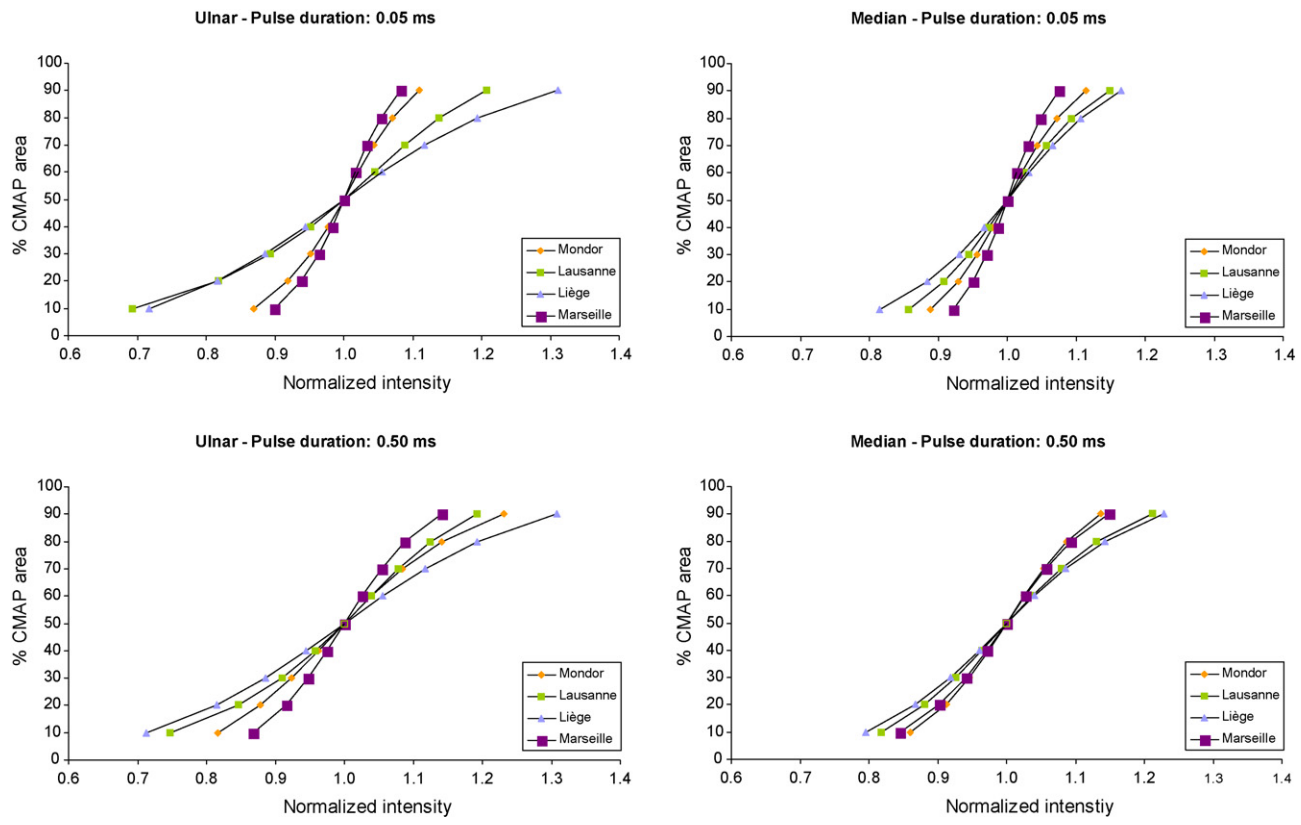

Figure 2 Averaged stimulus—response curves with normalization to I_{50} of the ulnar and median nerves stimulated at two different stimulus durations (0.05 and 0.5 ms) in the four centers of investigation.

centers regarding either the whole set of data ($p = 0.8708$, KW test) or any specific parameter ($p > 0.05$ in all cases).

In each center, we also compared the whole sets of data provided by median nerve versus ulnar nerve stimulation. A significant difference was found according to the stimulated nerve in Liège ($p < 0.0001$, Wilcoxon matched-pairs signed-ranks test) but not in the other centers ($p = 0.06$, 0.25 and 0.82 in Créteil, Lausanne, and Marseille, respectively). In Liège, the particularly low intensity thresholds observed for median nerve stimulation explained the difference found between data provided by median nerve versus ulnar nerve stimulation.

Comparisons between the centers

Between-center differences were significant regarding most of the studied parameters, particularly at 0.05 ms stimulus duration ($p < 0.005$, KW test) (Table 2). Between-center differences were not significant ($p > 0.005$) only for eight of the 40 analyzed parameters at 0.5 ms stimulus duration in all cases. These parameters were I_{10}, I_{50}, I_{90}, and I_{90}/I_{50} for ulnar nerve stimulation and I_{10}/I_{50}, $[I_{50} - I_{10}]/I_{10}$, and slope estimates for median nerve stimulation. After discarding the data from Liège because of the unexpected influence of the type of stimulated nerve on the results (see above), between-center differences were no more significant for the following parameters: I_{10}, I_{50}, and I_{90} for median nerve stimulation at 0.5 ms stimulus duration and I_{90}/I_{50}, $[I_{90} - I_{10}]/I_{10}$, and $[I_{90} - I_{50}]/I_{50}$ for median nerve stimulation at both stimulus durations.

In addition, between-center differences were significant for skin temperature and resistance at stimulation site (temperature: $p < 0.0003$; resistance: $p < 0.0001$, KW test), but not for the age and sex of the subjects enrolled in this study (age: $p = 0.0834$, KW test; sex: $p = 0.4753$, Chi-squared test).

Correlation studies were performed to assess the influence of skin temperature or resistance on stimulus—response curve data. Detailed results are presented in Table 4. Briefly, skin temperature correlated to intensity thresholds at 0.05 ms stimulus duration and to ''normalized'' slope estimates and ''Brismar-type'' ratios at both stimulus durations, while skin resistance correlated only to intensity thresholds at 0.05 ms stimulus duration.

Discussion

The goal of this study was to assess the reliability of various indexes provided by the stimulus—response curves in a multicenter clinical setting. First, it must be emphasized that this neurophysiological testing was easy to perform, rapid and well tolerated by all the subjects. Within-center assessments clearly showed that only two parameters, the ''normalized'' ratios I_{10}/I_{50} and I_{90}/I_{50}, were highly constant and reproducible. The results were more heterogeneous and conflicting regarding between-center comparisons. Several parameters did not vary significantly among the centers whatever the stimulated nerve, in particular at 0.5 ms stimulus duration and after discarding the data from Liège.

Table 2 Mean values (S.D.) of intensity thresholds (in mA), ratios, and slope estimates from the stimulus—response curves of the ulnar and median nerves stimulated at two different stimulus durations (0.05 and 0.5 ms).

	Créteil	Lausanne	Liège	Marseille
Ulnar 0.05 ms				
I_{10}***	46.53 (14.04)	28.73 (13.44)	15.79 (6.65)	17.38 (5.40)
I_{50}***	53.88 (17.12)	40.44 (16.20)	21.92 (7.59)	19.20 (5.41)
I_{90}***	59.58 (18.43)	48.59 (18.81)	28.95 (11.04)	20.61 (5.40)
I_{10}/I_{50}***	0.87 (0.06)	0.69 (0.14)	0.72 (0.17)	0.90 (0.06)
I_{90}/I_{50}***	1.11 (0.04)	1.21 (0.07)	1.31 (0.20)	1.08 (0.07)
$(I_{90}-I_{10})/I_{10}$***	0.28 (0.10)	0.83 (0.50)	1.01 (0.86)	0.21 (0.16)
$(I_{90}-I_{50})/I_{50}$***	0.11 (0.04)	0.21 (0.07)	0.31 (0.20)	0.08 (0.07)
$(I_{50}-I_{10})/I_{10}$***	0.16 (0.09)	0.51 (0.39)	0.51 (0.57)	0.12 (0.08)
Slope estimates***	0.10 (0.03)	0.06 (0.02)	0.12 (0.08)	0.47 (0.28)
Normalized slope estimates***	4.88 (1.43)	2.42 (1.01)	2.22 (0.90)	8.89 (5.81)
Ulnar 0.5 ms				
I_{10} (ns)	6.97 (2.09)	9.22 (4.73)	5.03 (2.10)	5.92 (1.83)
I_{50} (ns)	8.56 (2.64)	12.04 (5.55)	6.94 (2.33)	6.77 (1.93)
I_{90} (ns)	10.47 (2.97)	14.15 (6.01)	9.04 (3.01)	7.63 (1.97)
I_{10}/I_{50}**	0.82 (0.03)	0.75 (0.10)	0.71 (0.12)	0.87 (0.04)
I_{90}/I_{50} (ns)	1.23 (0.08)	1.19 (0.06)	1.31 (0.15)	1.14 (0.08)
$(I_{90}-I_{10})/I_{10}$**	0.51 (0.13)	0.61 (0.23)	0.91 (0.52)	0.32 (0.15)
$(I_{90}-I_{50})/I_{50}$**	0.23 (0.08)	0.19 (0.06)	0.31 (0.15)	0.14 (0.08)
$(I_{50}-I_{10})/I_{10}$**	0.23 (0.04)	0.36 (0.19)	0.46 (0.36)	0.15 (0.05)
Slope estimates***	0.31 (0.12)	0.19 (0.08)	0.28 (0.14)	0.56 (0.14)
Normalized slope estimates**	2.43 (0.37)	2.05 (0.75)	1.74 (0.56)	3.68 (1.19)
Median 0.05 ms				
I_{10}***	37.66 (9.51)	26.40 (9.67)	8.91 (2.64)	18.03 (4.65)
I_{50}***	42.17 (9.42)	31.28 (12.54)	10.78 (2.44)	19.51 (4.85)
I_{90}***	46.98 (10.48)	36.53 (16.20)	12.58 (3.08)	20.88 (4.88)
I_{10}/I_{50}*	0.89 (0.05)	0.86 (0.04)	0.81 (0.13)	0.92 (0.03)
I_{90}/I_{50}*	1.11 (0.02)	1.15 (0.08)	1.17 (0.05)	1.08 (0.04)
$(I_{90}-I_{10})/I_{10}$*	0.26 (0.08)	0.35 (0.17)	0.49 (0.41)	0.17 (0.07)
$(I_{90}-I_{50})/I_{50}$*	0.11 (0.02)	0.15 (0.08)	0.17 (0.05)	0.08 (0.04)
$(I_{50}-I_{10})/I_{10}$*	0.13 (0.07)	0.17 (0.06)	0.27 (0.31)	0.09 (0.04)
Slope estimates***	0.12 (0.04)	0.18 (0.13)	0.34 (0.13)	0.44 (0.17)
Normalized slope estimates*	5.00 (1.03)	4.29 (1.50)	3.56 (1.23)	8.45 (4.17)
Median 0.5 ms				
I_{10}***	5.44 (1.25)	7.90 (4.66)	2.53 (0.54)	5.01 (1.75)
I_{50}***	6.34 (1.60)	9.83 (6.14)	3.17 (0.54)	5.91 (2.00)
I_{90}***	7.22 (1.83)	11.93 (7.26)	3.88 (0.62)	6.74 (2.21)
I_{10}/I_{50} (ns)	0.86 (0.03)	0.82 (0.04)	0.80 (0.07)	0.84 (0.05)
I_{90}/I_{50} *	1.14 (0.03)	1.21 (0.07)	1.23 (0.07)	1.15 (0.07)
$(I_{90}-I_{10})/I_{10}$*	0.32 (0.05)	0.49 (0.13)	0.56 (0.20)	0.36 (0.13)
$(I_{90}-I_{50})/I_{50}$*	0.14 (0.03)	0.21 (0.07)	0.23 (0.07)	0.15 (0.07)
$(I_{50}-I_{10})/I_{10}$ (ns)	0.16 (0.04)	0.22 (0.05)	0.27 (0.14)	0.19 (0.06)
Slope estimates (ns)	0.56 (0.17)	0.37 (0.27)	0.75 (0.20)	0.56 (0.19)
Normalized slope estimates (ns)	3.30 (0.57)	2.53 (0.57)	2.35 (0.61)	3.15 (1.06)

Significance of the analysis of variance between the centers (Kruskal-Wallis test with Bonferroni correction): ns: $p > 0.005$; *: $p < 0.005$; **: $p < 0.001$; ***: $p < 0.0001$.

There was no clear explanation for the difference in the results provided by median nerve versus ulnar nerve stimulation in this center. On the whole, the absolute intensity thresholds (I_{10}, I_{50}, I_{90}) and the ratio I_{90}/I_{50} did not show significant differences between the centers. Considering the CoVs of these various indexes, only the ratio I_{90}/I_{50} showed high constancy in both within- and between-center analyses.

Most of the studied parameters appeared very variable among the centers. Technical considerations could easily explain this variability, such as differences in the type of EMG machine or electrodes used for stimulation and recording or in skin temperature or resistance at stimulation site. Although ranges of skin temperature and resistance have been defined initially, mean values varied significantly among the centers indicating that these ranges

Table 3 Mean coefficients of variation (CoV) and reproducibility (CoR) of intensity thresholds, ratios, and slope estimates from the stimulus—response curves of the ulnar and median nerves stimulated at two different stimulus durations (0.05 and 0.5 ms).

	Créteil		Lausanne		Liège		Marseille
	CoV	CoR	CoV	CoR	CoV	CoR	CoV
Ulnar 0.05 ms							
I_{10}	0.30	16.22	0.47	24.65	0.43	21.44	0.31
I_{50}	0.32	13.58	0.40	19.22	0.35	19.26	0.28
I_{90}	0.31	12.26	0.39	20.39	0.38	18.32	0.26
I_{10}/I_{50}	0.07	3.95	0.20	7.06	0.24	8.26	0.07
I_{90}/I_{50}	0.03	4.43	0.06	3.22	0.15	6.57	0.07
$(I_{90}-I_{10})/I_{10}$	0.36	30.07	0.60	20.73	0.85	29.15	0.74
$(I_{90}-I_{50})/I_{50}$	0.35	36.83	0.35	20.22	0.64	26.53	0.85
$(I_{50}-I_{10})/I_{10}$	0.56	29.25	0.77	23.51	1.11	32.45	0.70
Slope estimates	0.34	25.14	0.37	19.28	0.68	30.26	0.61
Normalized slope estimates	0.29	27.91	0.42	15.12	0.41	21.76	0.65
Ulnar 0.5 ms							
I_{10}	0.30	16.19	0.51	28.88	0.42	14.56	0.31
I_{50}	0.31	15.28	0.46	25.44	0.34	14.58	0.29
I_{90}	0.28	14.70	0.42	24.12	0.33	16.75	0.26
I_{10}/I_{50}	0.03	3.51	0.13	6.84	0.18	5.89	0.05
I_{90}/I_{50}	0.06	5.35	0.05	3.31	0.11	4.85	0.07
$(I_{90}-I_{10})/I_{10}$	0.25	26.42	0.38	21.50	0.57	20.55	0.48
$(I_{90}-I_{50})/I_{50}$	0.34	32.32	0.30	19.96	0.48	20.07	0.60
$(I_{50}-I_{10})/I_{10}$	0.18	20.40	0.54	28.51	0.79	21.65	0.34
Slope estimates	0.37	20.40	0.42	23.40	0.51	20.81	0.24
Normalized slope estimates	0.15	17.97	0.37	20.83	0.32	15.94	0.32
Median 0.05 ms							
I_{10}	0.25	17.85	0.37	14.52	0.30	22.15	0.26
I_{50}	0.22	16.67	0.40	16.39	0.23	16.11	0.25
I_{90}	0.22	18.11	0.44	17.79	0.24	16.40	0.23
I_{10}/I_{50}	0.06	4.49	0.05	2.29	0.16	7.04	0.03
I_{90}/I_{50}	0.01	2.09	0.07	3.14	0.05	2.45	0.03
$(I_{90}-I_{10})/I_{10}$	0.33	23.42	0.49	17.45	0.83	23.64	0.43
$(I_{90}-I_{50})/I_{50}$	0.15	18.04	0.57	23.86	0.33	17.98	0.47
$(I_{50}-I_{10})/I_{10}$	0.57	34.11	0.37	14.41	1.13	28.55	0.42
Slope estimates	0.29	25.95	0.73	26.09	0.39	22.69	0.40
Normalized slope estimates	0.21	17.99	0.35	17.13	0.35	18.27	0.49
Median 0.5 ms							
I_{10}	0.23	15.41	0.59	16.67	0.21	18.61	0.35
I_{50}	0.25	18.15	0.62	18.95	0.17	15.84	0.34
I_{90}	0.25	20.97	0.61	21.09	0.16	15.03	0.33
I_{10}/I_{50}	0.03	5.73	0.04	4.48	0.09	5.92	0.05
I_{90}/I_{50}	0.03	5.03	0.06	4.01	0.06	2.63	0.06
$(I_{90}-I_{10})/I_{10}$	0.15	31.99	0.27	22.27	0.36	23.29	0.35
$(I_{90}-I_{50})/I_{50}$	0.24	29.83	0.34	22.43	0.30	14.90	0.45
$(I_{50}-I_{10})/I_{10}$	0.25	32.53	0.24	24.20	0.52	28.19	0.34
Slope estimates	0.30	34.73	0.73	35.15	0.27	27.78	0.34
Normalized slope estimates	0.17	27.65	0.23	19.16	0.26	22.20	0.34

were too large. Our goal was to perform the test as in everyday practice of clinical electrodiagnosis. Obviously, inter-laboratory variation in the measurement of peripheral excitability could have been anticipated following a more strict protocol, independently from the use of different devices and electrodes. This should be taken into consideration in any future multicentric investigation of these parameters.

The correlation analyses performed in this study suggest that differences between the centers in skin temperature and resistance have impacted on the variability of several parameters of the stimulus—response curve. However, this influence was not significant for the absolute intensity thresholds (I_{10}, I_{50}, I_{90}) measured at stimulus duration of 0.5 ms and for the ''non-normalized'' slope estimates or the ''normalized'' ratios I_{10}/I_{50} and I_{90}/I_{50} at both

Table 4 Correlation between intensity thresholds, ratios or slope estimates on the one hand, and skin temperature or resistance at stimulation site on the other hand.

	Temperature	Resistance
Stimulus duration 0.05 ms		
Intensity thresholds (I_{10}, I_{50}, I_{90})	0.0056 (−0.55)	0.0382 (−0.43)
Normalized ratios (I_{10}/I_{50}, I_{90}/I_{50})	0.9824	0.9563
Brismar-type ratios [$(I_{90}-I_{10})/I_{10}$, $(I_{90}-I_{50})/I_{50}$, $(I_{50}-I_{10})/I_{10}$]	0.0013 (0.67)	0.4972
Slope estimates	0.9768	0.1710
Normalized slope estimates	0.0368 (−0.76)	0.4618
Stimulus duration 0.5 ms		
Intensity thresholds (I_{10}, I_{50}, I_{90})	0.6835	0.6853
Normalized ratios (I_{10}/I_{50}, I_{90}/I_{50})	0.8859	0.8650
Brismar-type ratios [$(I_{90}-I_{10})/I_{10}$, $(I_{90}-I_{50})/I_{50}$, $(I_{50}-I_{10})/I_{10}$]	0.0310 (0.44)	0.4359
Slope estimates	0.6646	0.9768
Normalized slope estimates	0.0368 (−0.75)	0.5364

The *p* values of the Spearman test are indicated (with correlation coefficient within brackets in case of statistical significance).

stimulus durations. In a previous study of motor nerve excitability properties in humans, the slope of the raw stimulus–response curve was also found to be unaffected by temperature variations [6]. Finally, stimulus duration appeared as an important factor of variability since the results were clearly more variable for shorter than larger stimulus duration. At 0.05 ms stimulus duration, the strength-duration curve of a motor nerve is very steep, and therefore, slight variations of intensities are likely to induce great changes in motor responses.

Whatever its causes, between-center variability is not a crucial problem because each laboratory has to establish its own normative data for the stimulus–responses curves as for any other electrophysiological method. Eventually, corrections for age and temperature can be applied from large normative data, as done by others in nerve excitability studies performed in patients [8]. In this way, the value of the present study was to show the low variability and good reproducibility of the ratios I_{10}/I_{50} and I_{90}/I_{50}. The fact that ratios with normalization to I_{50} provided less variable and more reproducible results than absolute values of threshold intensity was obviously expected but is clearly expressed in Fig. 2. The ratios I_{10}/I_{50} and I_{90}/I_{50} are able to assess separately the least and the most excitable fibers, and this may be interesting on clinical grounds because nerve fibers can be selectively affected in peripheral neuropathies, according to their diameter and excitability properties. Therefore, the ratios I_{10}/I_{50} and I_{90}/I_{50} should be preferred to the ratio $(I_{90}-I_{10})/I_{10}$ that was initially proposed by Brismar [2].

Such a low variability and good reproducibility should authorize the use of the ratios I_{10}/I_{50} and I_{90}/I_{50} of the stimulus–response curve in clinical neurophysiological practice, once normative data have been established. As mentioned in the introduction, the analysis of the stimulus–response curves can provide relevant information on axonal membrane properties related to nodal or internodal changes in nerve capacitance due to edema or demyelination. Such information is complementary to the information provided by standard nerve conduction studies regarding axonal loss and demyelination. In addition, this technique is rapid and nonpainful. However, the sensitivity and specificity of the proposed parameters in order to differentiate pathological from normal conditions remain to be established.

References

[1] Boërio D, Hogrel JY, Créange A, Lefaucheur JP. A reappraisal of various methods for measuring motor nerve refractory period in humans. Clin Neurophysiol 2005;116:969–76.
[2] Brismar T. Changes in electrical threshold in human peripheral neuropathy. J Neurol Sci 1985;68:215–23.
[3] Burke D, Kiernan MC, Bostock H. Excitability of human axons. Clin Neurophysiol 2001;112:1575–85.
[4] Cappelen-Smith C, Kuwabara S, Lin CS, Mogyoros I, Burke D. Membrane properties in chronic inflammatory demyelinating polyneuropathy. Brain 2001;124:2439–47.
[5] Kiernan MC, Burke D, Andersen KV, Bostock H. Multiple measures of axonal excitability: a new approach in clinical testing. Muscle Nerve 2000;23:399–409.
[6] Kiernan MC, Cikurel K, Bostock H. Effects of temperature on the excitability properties of human motor axons. Brain 2001;124:816–25.
[7] Kiernan MC, Guglielmi JM, Kaji R, Murray NM, Bostock H. Evidence for axonal membrane hyperpolarization in multifocal motor neuropathy with conduction block. Brain 2002;125:664–75.
[8] Krishnan AV, Kiernan MC. Altered nerve excitability properties in established diabetic neuropathy. Brain 2005;128:1178–87.
[9] Kuwabara S, Ogawara K, Sung JY, Mori M, Kanai K, Hattori T, et al. Differences in membrane properties in axonal and demyelinating Barré-Guillain syndromes. Ann Neurol 2002;52:180–7.
[10] Meulstee J, Darbas A, van Doorn PA, van Briemen L, van der Meche FG. Decreased electrical excitability of peripheral nerves in demyelinating polyneuropathies. J Neurol Neurosurg Psychiatry 1997;62:398–400.
[11] Ruijten MWMM, Salle HJA, Kingma R. Comparison of two techniques to measure the motor nerve refractory period distribution. Electroencephalogr Clin Neurophysiol 1994;93:299–305.
[12] Vucic S, Kiernan MC. Axonal excitability properties in amyotrophic lateral sclerosis. Clin Neurophysiol 2006;117:1458–66.